PHENOTYPIC PROPERTIES OF ENAMEL IN MOLAR-INCISOR HYPOMINERALISATION (MIH) AND AMELOGENESIS IMPERFECTA (AI) TEETH

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DECLARATION OF WORK

I, Mazlina Mohd Noor confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been acknowledged and indicated in the thesis.

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ABSTRACT

Background

Enamel is an external layer of the crown, and its production can be affected by genetic, systemic or environmental causes, leading to the formation of developmental defect of Enamel (DDE). Molar incisor Hypomineralisation (MIH) can be defined as a qualitative defect of systematic origin of the enamel, involving one or more first permanent molar, which is frequently associated with affected incisors. Amelogenesis Imperfecta (AI) is genetic condition, affecting the structure and clinical appearance of the enamel of all or nearly all the teeth in a more or less equal manner, and which may be associated with morphologic or biological changes elsewhere in the body. Knowledge about the chemical and mechanical properties of both conditions is beneficial to predict the severity and to provide appropriate treatment for individual patients.

Aim and Objectives

The aim of this study is to characterise the phenotypic properties of MIH and AI teeth in primary and permanent teeth. The objectives were to assess: tooth colour, radiographic features, hardness (on and off the anomaly), chemical variation and the ultrastructure of affected enamel vs. normal enamel.

Material and Methodology

Ethical approval was obtained. Twelve control, 12 MIH and 7 AI teeth were collected. The phenotype (DDE Index) for each sample was recorded. Prior to characterisation, the teeth were debrided and stored in 0.1% thymol at 4°C. Colour was examined using a spectrophotometer (SpectroShadeTM Micro) to quantify the variation in colour as defined by ΔE. Wallace indentation (H.M Wallace, Croydon, England) was also performed to assess the hardness value, denoted by Vickers Hardness Number (VHN). Raman microspectroscopy (HR 800, Jobin Yvon, Horiba, Japan) was used to study the chemical variation for each sample. Finally, the samples were sectioned at specific affected sites to image the ultrastructure of the enamel layer using a scanning electron microscopy (FEI XL30 FEGSEM (FEI UK, UK).

Results

All MIH and AI teeth showed various degrees of discolouration, with ΔE ranging from 2.47 for white/cream to 22.2 for yellow/brown defects. The range of enamel hardness of control teeth was 226.2 - 360.3 VHN. Meanwhile, enamel hardness for MIH and AI teeth was significantly reduced (P = 0.004) ranging from 13.1 - 142.6 VHN. Chemically, both MIH and AI teeth showed a significant increase in carbonate to phosphate ratio when studied under Raman spectroscopy (P = 0.000). Histologically, the affected hypomineralised enamel appeared more porous, disorganised, with loss of enamel rod

structure and presence of structureless layer compared the normal enamel.

Conclusion

Teeth diagnosed with MIH and AI showed marked discolouration and lower hardness values compared with normal enamel. Yellow/brown opacities had lower hardness values than white/cream opacities. These conditions also caused changes in the chemical properties and histological structure of the enamel.

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TABLE OF CONTENT

LIST OF FIGURE

LIST OF TABLE

ABREVIATIONS

AD	Autosomal Dominant
AFM	Atomic Force Microscopy
AI	Amelogenesis Imperfecta
AMBN	Ameloblastin
AMELX	Amelogenin
AR	Autosomal Recessive
BW	Bitewing
CEJ	Cementum Enamel Junction
DDE	Developmental Defect of Enamel
DEJ	Dentine Enamel Junction
DPT	Dental Panoramic Tomography
DSPP	Dentine Sialophosphoprotein
ENAM	Enamelin
GPa	Giga Pascal
HeNe	Helium-Neon
HSB	Hunter Schreger Bands
ICD	International Classification of Disease
IEE	Inner Enamel Epithelium
KHN	Knoop Hardness Number
KLK4	Kallikrein 4
LCH	Light, Chroma and Hue
PEB	Post Eruptive Breakdown
LL3	Lower left permanent canine
LL6	Lower left permanent first molar
LR4	Lower right first premolar
LR6	Lower right permanent first molar
MIH	Molar Incisor Hypomineralisation
MMP20	Matrix Metalloproteinase 20
OCT	Optical Coherence Tomography
OEE	Outer Enamel Epithelium
PA	Periapical
POLMI	Polarised Light Microscope
REE	Reduced Enamel Epithelium
SEM	Scanning Electron Microscopy
TDO	Tricho Dento Osseous

TUFT1	Tuftelin
UL4	Upper left first premolar
UL5	Upper left second premolar
UL6	Upper left permanent first molar
ULA	Upper left primary central incisor
ULE	Upper left primary second molar
UR4	Upper right first premolar
UR6	Upper right permanent first molar
URE	Upper right primary second molar
USO	Upper Standard Occlusal
VHN	Vickers Hardness Number

CHAPTER 1
INTRODUCTION

1 INTRODUCTION

1.1 Statement of problem

Molar Incisor Hypomineralisation (MIH) and Amelogenesis Imperfecta (AI) are two types of anomalies that affect the structure and appearance of the enamel of teeth. Both conditions can lead to various problems for affected patients, and can be difficult for the dentist to manage. The problems that may arise include:

Patient Related:

- Aesthetic concerns
- Sensitivity and discomfort
- Fragility and porosity
- Post-eruptive breakdown (PEB) and subsequent caries formation

Treatment Related:

- Bonding of restorative materials
- Difficult to distinguish between MIH and AI in early mixed dentition phase
- Difficult to anaesthetise

In MIH, the enamel undergoes chronological hypomineralisation during its formation caused by several factors (Fagrell, 2011). It always affects the first permanent molars, and may also affect the incisors. Clinically, the enamel shows white/cream to yellow/brown opacity. The defective enamel also has a high risk of having PEB soon after the tooth eruption that can lead to dentinal exposure. Patient may experience hypersensitivity that can cause difficulty in maintaining good oral hygiene care and avoidance of certain foods. Treatment of MIH is dependent on the severity and also the symptoms experienced by the patients.

AI is a genetic condition that develops due to mutation of the genes involved in the formation of enamel. It can be classified as hypoplastic, hypocalcification and hypomaturation and can affect both the primary and permanent dentition. The enamel of the affected teeth may appear small, pitted, discoloured and prone to wear. Patients may also complain of sensitivity due to the exposed dentinal layer. Management of AI is a lifelong process and can vary from managing the sensitivity to restoring the affected teeth in order to maintain the function as well as the aesthetic appearance.

Therefore, knowledge and understanding about the chemical and mechanical properties of MIH and AI is beneficial in order to anticipate the severity and behaviour

of the conditions so that the most appropriate treatment option can be proposed for the patient's best interest.

In this study, our main objective is to describe and to explore the enamel defects in terms of the type of defect, colour, hardness, chemical variation and ultrastructure characteristics.

CHAPTER 2
REVIEW OF LITERATURE

2 REVIEW OF LITERATURE

2.1 Introduction

Developmental dental anomalies often exhibits patterns associated with the developmental stage at which the malformations occurred.. Dental anomalies can affect tooth number (i.e supernumerary teeth and hypodontia), size (i.e macrodontia and microdontia), shape (i.e dens evaginatus) or structure (i.e AI, MIH and fluorosis) (Pinkham et al., 2005).

Enamel defects can occur at any stage of tooth development and are usually associated with either local factors such as trauma and infection or general factors (i.e genetic, systemic), although sometimes their aetiology is unknown.

Enamel defects can be attributed to a number of factors related to the child, mother and socio-demographic factors. A significant association between children's nutritional status, very low birth weight child and developmental defects of enamel has been demonstrated (Correa-Faria et al., 2013). Children with a history of premature birth have a greater chance of developing enamel defects in the primary dentition (Lunardelli and Peres, 2005).

The common enamel defects among children is MIH, which is described as qualitative defect, which can be identified visually. The defective enamel may be white, brown or yellow. MIH has been reported since early 1970's where developmental defects were noted mainly located on the first molars and incisors of the permanent dentition (Fagrell, 2011).

One of the most prominent studies regarding the prevalence of demarcated opacities in first permanent molar and incisor was carried out in 1977 (Koch et al., 1987). This epidemiological study focused on the prevalence, extension and severity of idiopathic enamel hypomineralisation in children born in 1970 (+/- 1 year). They found that in general, more than 15% of the children born in 1970 showed hypomineralised enamel compared to the children born before and after that year. The remarkable result in children born in 1970 has been described as possible specific influence on the development of enamel during that time period. When the study was conducted, the Developmental Defect of Enamel (DDE) index was not available. So, they developed a system to record the hypomineralised enamel, using the smooth surfaces, as the fissure was difficult for differential diagnosis. It was divided into three units; incisal /

occlusal, intermediate and gingival units. Each unit was examined according to the colour and surface changes only. In order to be considered as enamel hypomineralisation, the changes of the colour or structure must involve more than one third of a tooth unit. There was also evidence that if a mandibular incisor was affected, the maxillary incisors and any molars usually showed hypomineralisation as well.

Enamel defects can also be due to hereditary or genetic causes, such as AI, which is defined as “a group of conditions, genomic in origin, which affect the structure and clinical appearance of the enamel of all or nearly all the teeth and which may be associated with morphologic or biochemical changes elsewhere in the body” (Aldred et al., 2003). An early classification for hereditary enamel defects divided them into 2 categories; enamel hypoplasia and enamel hypocalcification (Crawford et al., 2007). The mode of inheritance can be autosomal dominant (AD), autosomal recessive (AR), X-linked or sporadic. Furthermore, there can be an association with several syndromes for example tricho-dento-osseous syndrome (TDO), cone-rod dystrophy and nephrocalcinosis (Crawford et al., 2007, Michaelides et al., 2004, Lubinsky et al., 1985).

Teeth affected with AI and MIH can cause problems such as hypersensitivity, discomfort, high risk of developing caries, enamel fragility and porosity and aesthetic concerns. It can be difficult for the dentist to distinguish between AI and MIH without a clear history, as the clinical presentations may be similar in the early mixed dentition. In addition, teeth affected with enamel defects can be difficult to anaesthetise and restore. Patients with severe MIH affecting the first permanent molars also may demonstrate dentist fear, concern and reluctant to have the treatment due to the hypersensitivity (Jalevik and Klingberg, 2002).

There have been several studies investigating the properties of enamel in MIH and/or AI. Most studies have assessed enamel hardness, microstructure and also chemical variations in MIH and AI primary or permanent teeth. However, most of the studies have focused on the permanent rather than primary teeth.

A study carried out in Brazil in 2003 showed that almost one quarter of the examined pre school children aged 3 to 5 years old, presented with developmental defect of the enamel in their primary dentition. The most common affected teeth were the second molars followed by the first molars (Lunardelli and Peres, 2005). Another study showed 29.9% suffered from developmental defect of enamel in primary teeth among the pre

school children (Correa-Faria et al., 2013). This indicates that the prevalence of enamel defect in primary teeth is quite high, although studies investigating the properties in primary teeth is still sparse. This is maybe due to the difficulty to obtain the primary tooth sample in such young children as most of the teeth usually being restored with the restorative material to preserve the structure prior to the eruption of the permanent successor.

In many cases of patients with AI, the primary teeth are less affected than the permanent teeth and the reason for this is not known. Increasing our knowledge of how the physical properties are affected in primary teeth is therefore important.

To explore the ways that enamel defects can occur, we first need to consider how normal enamel formation and development occurs.

2.2 Tooth Formation

A tooth originates from the ectoderm and mesoderm. The formation starts at approximately 6 weeks of age intrauterine. The oral epithelium thickens and invaginates into the ectomesenchyme to form the primary epithelial band. This band later divides into two processes; the buccally located vestibular lamina and the lingually situated dental lamina. The vestibular lamina is responsible for the formation of vestibule of the mouth, delineating the lips and cheeks from the tooth-bearing region. The dental lamina contributes to the development of teeth. All of the primary teeth originate from the dental lamina, while the permanent successors arise from its lingual extension and the permanent molars from its distal extension (Nanci, 2013). The dental lamina multiplies at a faster rate at 10 specific intermittent locations along the basement membrane.

These 10 locations represent the 10 primary teeth for each arch (Pinkham et al., 2005). The tooth germ is an aggregation of cells that eventually forms a tooth. These cells are derived from the ectoderm of the first brachial arch and the ectomesenchyme of the neural crest (Nanci, 2013). The tooth germ consists of three distinct parts; the enamel organ, dental papilla and the dental sac or follicle.

The enamel organ (Figure 2-1) is composed of the outer enamel epithelium (OEE), inner enamel epithelium (IEE), stellate reticulum and stratum intermedium. The enamel organ is not only for the enamel formation, but also play an important role during the

initiation of dentine formation and establishment of the dentogingival junction. After enamel maturation, it becomes part of the reduced enamel epithelium (REE) (Nanci, 2013).

Figure 2-1Enamel organ.
Image courtesy of (Nanci, 2013).

The dental papilla comprises of the cells that produce dentine and pulp tissue called odontoblasts. It lies below the enamel organ. Furthermore, the junction between the dental papilla and IEE determines the crown shape of a tooth. Mesenchymal cells within the dental papilla are responsible for formation of the tooth pulp (Nanci, 2013).

The dental follicle is a sac containing the developing tooth and its odontogenic organ. The dental follicle gives rise to three important cells that form the periodontium including the cementum, periodontal ligament and alveolar bone. They are known as cementoblasts, fibroblasts and osteoblasts. Cementoblasts form the cementum of a tooth. Osteoblasts give rise to the alveolar bone around the roots of teeth. Fibroblasts are involved during the development of the periodontal ligament which connect teeth to the alveolar bone through cementum (Nanci, 2013).

Tooth development is usually divided into initiation, bud, cap and bell stages. The initiation stage is first noticed at the 6 weeks gestational age.

During the bud stage, the tooth buds multiply and expand further. The dental epithelium separates into two types of cell lineage, the peripheral basal cell and the stellate reticulum cell. These cells will further form the epithelial component in the continuously growing teeth (Thesleff and Tummers, 2008). Along with the formation of the dental lamina, 10 round epithelial structures, each referred to as a bud, develop at the distal aspect of the dental lamina of each arch. These correspond to the 10 primary

teeth of each dental arch, and they signify the bud stage of tooth development. The dental mesenchyme condenses around the bud and segregates into two cell lineages, the dental papilla which later becomes surrounded by dental epithelium and gives rise to the tooth pulp and dentin producing odontoblasts, and the peripheral dental follicle giving rise to the cementoblasts and periodontal tissues. The permanent tooth buds develop lingual to the primary ones. Disturbances in this stage will lead to hypodontia and supernumerary teeth (Nanci, 2013, Berkovitz et al., 2009).

The cap stage occurs by the 11th week intrauterine. Morphogenesis progresses, with the enamel organ invaginating to form a cap-shaped structure. At this stage, the size and shape for each tooth become more apparent, this process is regulated by the enamel knot. The signal from the enamel knots regulates the growth and determines the sites of the epithelial folds which correspond to the cusp pattern (Thesleff and Tummers, 2008). Later, the cap-shaped structure becomes the enamel organ covering the dental papilla and eventually producing enamel and dentine/pulp respectively.

The bell stage is also known as 'histodiffferentiation' and 'morfodifferentiation' stage. It starts by the 14th week of intrauterine age and is divided into early and late bell stage. At this stage the enamel organ is bell-shaped. Most of the cells are called stellate reticulum because of the star-shaped appearance. The main function of this cell is to protect the underlying dental tissue against the physical disturbance and also to maintain the tooth shape (Berkovitz et al., 2009).

The OEE is thought to be involved in the maintenance of the shape of the enamel organ. The cervical loop, situated at the margin of the enamel organ where the OEE is continuous with the IEE, has high mitotic activity. The IEE is first seen during the bell stage, when the cells differentiate into ameloblasts and produce the enamel matrix. Once the ameloblast cells are formed, the dental papilla begins to differentiate to form odontoblasts. Both ameloblast and odontoblast cells are responsible for the future development of enamel and dentine respectively.

Dentine formation commences when there is a thickening of the basement membrane of the IEE. The dentinal layer must be present for the enamel to form. However, the presence of ameloblasts are also essential in order for dentinogenesis to continue (Pinkham et al., 2005).

At this stage, the dental lamina starts to disintegrate as the developing tooth erupts into the oral cavity (Nanci, 2013). However, some adult may have remnants of the dental lamina as clumps of resting cells (epithelial of Serres) that can be involved in cyst formation (Berkovitz et al., 2009).

During the late bell stage (also known as oppositional stage), the formation of dental hard tissue commences. It occurs at the approximately 18th week of intrauterine age. Dentine formation precedes enamel formation. The development of permanent dentition is initiated via the lingual downgrowth of the OEE in primary teeth that will later form the tooth germ of the permanent successors. For those teeth, which are not replacing the primary teeth, the dental lamina of the primary second molar grows backwards to buds off successfully the permanent molar teeth. The first permanent molar appears at about 4 months in utero, the tooth bud for the second permanent molar appears about 6 months after birth, while the third permanent molar appears at about 4 to 6 years after birth (Berkovitz et al., 2009). The summary of tooth development is shown in Figure 2-2.

Figure 2-2 Stages of tooth development. Early development is directed at creating the crown and only then root formation is initiated. Ameloblasts differentiate from the epithelium and odontoblasts from the mesenchyme and they deposit the matrices of enamel and dentin, respectively. Ep, epithelium; mes, mesenchyme; sr, stellate reticulum; dm; dental mesenchyme; dp, dental papilla; df, dental follicle; ek, enamel knot; erm, epithelial cell rests of malassez; hers, hertwig's epithelial root sheath.
Image courtesy of (Thesleff and Tummers, 2008).

2.3 Enamel

Enamel is the hard, thin, translucent substance covering and protecting the dentine of the crown of a tooth, and is composed almost entirely of calcium salts. Enamel contains both inorganic and organic material. The inorganic material consists of crystalline calcium phosphate known as hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) crystals, which is over 95% of its volume. Hydroxyapatite tends to incorporate large number of trace element (Weatherell et al., 1974). One constituent that is incorporated into developing enamel is carbonate. It can be substituted to hydroxyl group (A-type carbonate) or phosphate group (B-type carbonate) (Sydney-Zax et al., 1991).

Enamel does not contain collagen, as is usually found in other hard tissue, such as dentine and bone. However, it does contain unique proteins called amelogenin and enamelin (Fincham et al., 2000). The amelogenin protein is the major component of the continuously secreted enamel extracellular matrix and enamelin is a minor component

of the matrix that control the mineralisation of enamel crystals (Iijima et al., 2010). The other proteins that involve in enamel formation includes amelogenin, ameloblastin, tuftelin, amelotin and dentine sialophosphoprotein (Crawford et al., 2007).

Studies have shown the presence of many other proteins in the enamel extracellular matrix, such as albumin, serine proteases, glycoconjugates and Calcium (Ca+) dependent proteases (Fincham et al., 2000). The role of these proteins is not fully understood but it is postulated that they support in the development of enamel by serving as a framework for minerals to form on.

In humans, enamel varies in thickness over the surface of the tooth, often thickest at the cusp (up to 2.5 mm) and thinnest at its border (about 1.3 mm) with the cementum at the CEJ (Nanci, 2013).

2.3.1 Amelogenesis

Amelogenesis is a process for the formation of enamel on teeth. This process occurs simultaneously with dentine formation (dentinogenesis) but as a distinctly different process. Although dentine must be present for enamel to form, ameloblasts must also be present in order for dentinogenesis to start. A message is sent from the newly differentiated odontoblasts to the IEE, causing the epithelial cells to further differentiate into active secretory ameloblasts. Dentinogenesis is in turn dependent on signals from the differentiating IEE in order for the process to continue. This interaction between the IEE and odontoblasts is an example of a biological concept known as reciprocal induction between the mesenchymal cells and epithelial cells.

Initially the enamel is secreted as a soft partially mineralized organic matrix, which comprises of 30% mineral only with the remainder being organic material and water. This ratio is gradually reversed and the mature tissue contains more minerals than the organic material (Robinson et al., 1995)

Enamel formation can be described as four stages; pre secretory, secretory, transition and maturation (Berkovitz et al., 2009).

2.3.1.1 Pre Secretory Stage

This stage is also known as the inductive stage where the morphodifferentiation of the

ameloblast takes place. During the bell stage of tooth formation, the IEE differentiates to form the enamel-forming cell known as the ameloblast. The differentiation begins at the future cusp or tip and progresses towards the cervical direction (Berkovitz et al., 2009). The ameloblast cells differentiate from cuboidal to columnar cells. These columnar cells are pre ameloblast, which have centralized nuclei and poorly developed Golgi complexes. At this time, the dentine is not mineralized.

After the changes in the IEE, the adjacent mesenchymal cells of the dental papilla start to differentiate into odontoblasts. Then, the basal lamina separating the two cells disappears as the first dentine layer is laid down and provides a signal for the ameloblast to begin secretion (Berkovitz et al., 2009).

2.3.1.2 Secretory Stage

In the secretory stage, the ameloblasts become long columnar cells with the nuclei located at the basal end, away from the forming enamel. Enamel matrix is deposited on the preformed dentine as the ameloblasts are pushed away from the dentine surface. Crystals of hydroxyapatite start to form immediately after the enamel matrix secretion (Robinson et al., 1995).

After the deposition of the initial, thin and aprismatic enamel, a cone-shaped or pyramidal-shaped process, “Tomes process” forms at the distal secretory end of the ameloblast. The shape of the Tomes processes is important for the formation of prismatic structure of enamel (Berkovitz et al., 2009).

The initial hydroxyapatite crystallites are thin and needle-like and much smaller than the crystallites found in mature enamel. Enamel crystallites form perpendicular to the distal surface of the ameloblast with each crystallite appearing as flattened hexagons when viewed in cross-section. The enamel crystallites that elongate around the tip of the Tomes process form the region of the enamel rod core. Crystallites extending from where the ameloblasts are joined to each other form the region called enamel rod boundary or inter rod enamel. There is a clear border between the enamel rod core and enamel rod boundary as part of the ameloblast is non-secretory. Four ameloblasts contribute to the formation of single enamel rod and each ameloblast is involved in the development of four rods (Berkovitz et al., 2009).

The secretory phase ends once the full thickness of the enamel matrix has been

secreted. The ameloblasts' secretory extension (Tomes process) disappears and the ameloblast distal end becomes flattened. The signalling mechanisms that determine when and where in the tooth the secretory process is turned on or off are unknown (Berkovitz et al., 2009).

2.3.1.3 Transition Stage

The initial enamel matrix that has been laid down during the secretory stage has a high content of water and protein, but low mineral content and is porous. The conversion of this young and immature enamel to well-mineralised enamel is called maturation. The period between the secretory stage and maturation stage is called the transition stage. At this stage, enamel secretion stops and much of the matrix is withdrawn. Towards the end of this stage, the crystallites size appears to increase, corresponding to an increase in the mineral content (Robinson et al., 1995).

2.3.1.4 Maturation Stage

During this stage, the enamel crystallites grow further and cause an increase in width and thickness. The ameloblast undergoes morphological changes as the Tomes process disappears, and the internal structure is totally rearranged and the length reduced by half (Robinson et al., 1995). Microscopically, these cells become striated, or have a ruffled border. These signs demonstrate that the ameloblasts have changed their function from production, as in the secretory stage, to transportation.

Ameloblasts transport calcium, phosphate and carbonate ions into the matrix and remove water and degraded enamel matrix protein from it. The overall mineral content of enamel rises abruptly at the beginning of maturation, due to mineral ions uptake which reaches as high as 95% mineral by volume (Robinson et al., 1995). It is also important to note that enamel mineralisation does not occur homogeneously, in thicker enamel, inner layer is often less well mineralised compared to the outer layer.

Once maturation is completed, the flattened ameloblast together with the remnant of the enamel organ constitutes the REE. Enamel mineralisation only occurs once, as ameloblasts are lost when a tooth erupts, within the REE; therefore after amelogenesis, enamel production has been finalised.

The mineralisation process of enamel or dentine is very sensitive, and takes place over a long period of time. The ameloblast cells has a limited reparative capacity, hence any disturbance during the mineralisation of enamel may result in permanent discolouration or a deficient enamel layer (Pinkham et al., 2005).

2.4 Properties of Enamel

2.4.1 Physical Properties

2.4.1.1 Colour

Enamel is a crystalline material that refracts light differently in different directions. The normal colour of enamel varies from light yellow to greyish (bluish) white. Young enamel colour is white, although, light enters it readily, almost all of it reflected with no absorption. This results in low translucency and the white colour. Tooth colour is influenced by a combination of intrinsic colour and the presence of extrinsic stains that may form on the tooth surface.

Light scattering and absorption within enamel and dentine give rise to the intrinsic colour of the teeth and since enamel is relatively translucent, the properties of dentine can play a major role in determining the overall tooth colour (Joiner, 2004). A study showed that the average scattering of normal tooth is 0.6 (± 0.4) mm^{-1} with enamel playing only a minor role through scattering at wavelengths in the blue range (ten Bosch and Coops, 1995).

At the edges of teeth where there is no dentine underlying the enamel, the colour sometimes has a slightly blue tone. Since enamel is semi translucent, the colour of dentine and any material underneath the enamel strongly affects the appearance of a tooth.

The enamel on primary teeth has a more opaque crystalline form and thus appears whiter than on permanent teeth. The translucency of enamel increases with age and some of the colour of the underlying dentine is then transmitted leading to the yellow appearance (Berkovitz et al., 2009).

2.4.1.2 Hardness

Enamel is the hardest biological tissue in the body and highly mineralised. It can withstand both shearing and impact forces. It also has high abrasion resistance to limit wear. These properties are very important as enamel can undergo neither repair nor replacement (Berkovitz et al., 2009).

Enamel hardness is one of the most widely studied between the other mechanical properties. The values of enamel hardness have been reported either by KHN or VHN depending on the method used. However, the value obtained from both methods showed no important differences (Gutierrez-Salazar and Reyes-Gasga, 2001).

The hardness of normal enamel in permanent teeth is between 2.71 – 4.15 GPa with a modulus of elasticity of between 62.06 – 95.77 GPa (Mahoney et al., 2004a, Mahoney et al., 2004b). One study measured the mechanical properties of a single enamel prism/rod in third permanent molars using atomic force microscopy (AFM), together with nano-indentation techniques to verify the structure. The mean hardness was 3.9 (± 0.3) and 3.3 (± 0.3) GPa when measured parallel and perpendicular to enamel rods respectively (Habelitz et al., 2001).

The enamel hardness remains constant from the outer enamel surface to the enamel dentinal junction (Gutiérrez-Salazar and Reyes-Gasga, 2003). A study combining Vickers indentation and SEM successfully demonstrated the differences of the mechanical properties of enamel (Xu et al., 1998). Fagrell *et al.* used the digital micro hardness tester fitted with a Vickers diamond to make an indentation on the enamel surface to measure the enamel hardness in normal and hypomineralised teeth. The results showed that the mean value for the hardness of the enamel in normal teeth was more than 2 times higher than the hypomineralised enamel which are VHN 350.7 and 144.3 respectively (Fagrell et al., 2010).

None of the above methods were able to measure the mechanical properties of single enamel rods. A study carried out by Habelitz *et al.* using the atomic force microscopy (AFM) together with a nano-indentation technique, to verify the structure and demonstrate mechanical testing of the enamel rod in third permanent molar. The mean hardness of 3.9 (± 0.3) and 3.3 (± 0.3) GPa were measured for parallel and perpendicular to enamel rods respectively (Habelitz et al., 2001).

2.4.1.3 Ultrastructure

The principle mineral component of enamel is calcium hydroxyapatite. It presents in a form of crystallites, which are hexagonal in shape in cross section. Millions of crystallites form enamel rod, the basic structural unit of enamel. The size for each crystallite is about 70 nm in width, 25 nm thick and extending across the full width of the tissue.

Enamel rods run from the enamel-dentine junction to the surface. In cross-section, the enamel rods appear as keyhole arrangements, with the tails pointing cervically and the heads occlusally. In the head of the rods, the crystallites run parallel to the long axis of the prism. In the tail, the crystallites gradually diverge 65°- 70° to the long axis (Berkovitz et al., 2009).

The enamel between the enamel rods known as inter rods. The composition is similar to that within the rod. Nonetheless, a histologic distinction is made between the two because the crystal orientation is different in each. The crystals in the inter rod enamel lie nearly perpendicular to the enamel rod.

In the longitudinal axis, most of the enamel rods appear to travel in a sinusoidal line from the DEJ to the surface. However there is periodic change in rods direction give rise to banding pattern called Hunter Schreger bands (HSB).

Hunter-Schreger bands

HSB is the term given to describe the arrangement of the enamel rods, where the enamel rods are arranged in layers of varying thickness at about right angles to each other. These bands are about 50μm wide. HSB strengthen the enamel and prevent crack from propagating through the tooth.

In sections of enamel cut parallel to the long axis of the tooth, the individual crystallites will be oriented differently in the groups of enamel rods cut more transversely or more longitudinally.

Cross Striation

Cross striations are the lines that cross the enamel rods at right angles to the long axis with common interval of about 3 – 6 μm. These are diurnal being formed every 24 hours parallel to the secretory face of the ameloblasts. The alternating constriction and

expansions of the rods represent the structural relationship between the rod and inter rod enamel (Nanci, 2013).

Striae of Retzius

The striae of Retzius are incremental growth lines or bands seen in enamel. It can be viewed if the enamel cut along the longitudinal axis of the crown and it run obliquely across the enamel rod to the surface (Berkovitz et al., 2009). When viewed microscopically in cross-section, they appear as concentric rings. In a longitudinal section, they appear as a series of dark bands. The presence of the dark lines is similar to the annual rings on a tree.

2.4.2 Chemical Properties

The composition between the crystallite core and the periphery is slightly different, as the core is rich with magnesium and carbonate, which makes it even more soluble than the periphery. It is known that carbonate ion can replace the phosphate or hydroxyl ions in hydroxyapatite. During the secretory stage of enamel formation, the concentration of magnesium and carbonate are relatively high. It is may be related to the less ordered young crystallites, and more extraneous materials. The inclusion of the carbonate form “carbonatoapatites” tends to produce less stable apatite (Robinson et al., 1995).

Fluoride is also incorporated into the hydroxyapatite crystallites forming fluoroapatite. Unlike carbonate and magnesium, fluorides enhance the crystallite structure to become more highly ordered. As secretion proceeds, the concentration of carbonate, magnesium and fluoride falls towards transitional stage. The crystallites grow and there is evidence of an increase in mineral content. At the end of maturation stage, the mineral content of the enamel rises steeply compared to the matrix composition (Robinson et al., 1995).

The remainder of the tissue is water and organic material. The presence of water is associated with the porosity of the tissue, which may lie between the crystal and surround the organic material.

During the early stage of enamel formation, the composition of the immature enamel matrix is protein and peptides. The developing enamel contains two types of protein; the amelogenins and non-amelogenins (enamelins). Following the maturation process,

the majority of the amelogenin proteins are degraded and removed and only the enamelin proteins remain in mature enamel.

2.5 Developmental Dental Defect

Developmental Dental Defect is a general term to describe disruption during the development of dental hard tissue including enamel and dentine. Unlike bone, enamel is not remodelled. Therefore, any disturbance in the ameloblast or odontoblast can cause a permanent defect. The defect can be either qualitative or quantitative or both.

The aetiology of developmental dental defect can be environmental or genetic. Environmental dental defect can be associated with systemic or local factor. Usually if the defect affected a single tooth or several neighbouring teeth considered as due to local factor. General systemic defect are related to the timing and sequence of the development of the teeth or also known as chronological defect. Genetic defect can be restricted to dental tissue or the dental tissue can be a manifestation of generalised tissue involvement.

The most common development dental defects in enamel are molar incisor hypomineralisation (MIH), Amelogenesis Imperfecta (AI) and fluorosis. Developmental dental defect in dentine includes dentinogenesis imperfecta and dentine dysplasia.

The awareness regarding developmental dental defect is increasing between dentist and patient, with a group of practitioners, scientist and public health representatives from Melbourne forming a group called D3 Group in 2007. The aim of this group is to increase awareness, improve understanding and cared for better with regards to developmental dental defect. The target groups to disseminate the information are families and patient, the public health sector, practitioner and industry, researchers, educators and students. The D3 website (http://thed3group.org) provides information especially for the researcher interested in MIH.

2.6 Molar Incisor Hypomineralisation

The term MIH was introduced in 2001 in order to describe the clinical presentation of hypomineralised enamel of systemic origin. It is also known as hypomineralised permanent first molars (PFM), idiopathic hypomineralisation, dysmineralised PFM and cheese molars. MIH occurs due to the disruption during the amelogenesis in transition and maturation stage (William et al., 2006). A study showed that the hypomineralised enamel had a mineral concentration gradient opposite to that of normal enamel and the pattern of mineral concentration suggests a disturbance during the maturation process (Suckling et al., 1989).

2.6.1 Definition

MIH is defined as “a qualitative defect of systematic origin of the enamel, involving one or more first permanent molar, which is frequently associated with affected incisors” (Weerheijm et al., 2001).

2.6.2 Epidemiology

The prevalence varies between countries where the study conducted. The prevalence of MIH among children were from 2.8% in Hong Kong (Cho et al., 2008), 22% in Spain (Garcia-Margarit et al., 2014) to 40% in Denmark and Brazil (Wogelius et al., 2008, Soviero et al., 2009). Any number between 1 and 4 of the first permanent molars may be affected in MIH. The more teeth affected, the higher the probability of more severe clinical presentations. About half of the children with MIH also had affected incisors, with a further third having severe hypomineralisation (Jalevik et al., 2001a).

A study showed the relationship between deciduous molar hypomineralisation (DMH), which usually refers to the primary second molar, and MIH suggests DMH can be used as a predictor for MIH. The study also indicated the prevalence of DMH among young children in the Netherlands was 9% (Elfrink et al., 2012).

2.6.3 Aetiology

The aetiology of MIH is still unknown. However, there were some possible causes that might be responsible for the development of MIH. In order to understand the aetiology of MIH, it is essential to know the normal process of enamel mineralisation in terms of the chronology of tooth mineralisation. Mineralisation of the enamel of the first

permanent molars start just before birth and is completed at the age of 3 years (Reid and Dean, 2006).

Several studies have suggested various factors contributing to MIH, those including; hypoxia (Lygidakis et al., 2008), hypocalcaemia (Jalevik et al., 2001a) and preterm birth (Brogardh-Roth et al., 2011). Other studies proposed a correlation between MIH and exposure to dioxins via mother's breast milk especially in prolonged breastfeeding (Alaluusua et al., 1996a, Alaluusua et al., 1996b).

Researchers have also looked at the feeding patterns and toxins in the environment during the postnatal period (Alaluusua et al., 1996a, Alaluusua et al., 1996b). Other probable causes suggested include underlying diseases in childhood such as chicken pox, fever and various types of medications such as antibiotics (Jalevik et al., 2001b).

A systematic review looking at fifty three articles, assessed the strength of evidence for the aetiology of MIH, and found five factors implicated in the aetiology of MIH (Crombie et al., 2009):

1. Exposure to environmental contaminants such as polychlorinated biphenyls and polychlorinated dibenzo-p-dioxin / dibenzofurans (dioxins)
2. Pre, peri and neonatal problems
3. Fluoride exposure
4. Common childhood illness
5. Medically compromised population

However, the evidence provided by the majority of the papers was low, and it was concluded that future studies are needed to establish the aetiological factors that contribute to the formation of MIH, by improving the study design as well as the standardisation of diagnostic and examination protocols.

A recent study suggested that MIH might have a multifactorial aetiology including prematurity, gastrointestinal problems, pneumonia, frequent high fever, measles and chicken pox before the age of four. It appears that rather than just the first year of life, the first four years of life are important for the development of MIH (Sonmez et al., 2013).

2.6.4 Clinical Presentation

Hypomineralised enamel can be described as an abnormality in the translucency of the enamel (opacity), and in MIH the lesion is usually demarcated in contrast to diffuse opacities which is a typical finding in fluorosis (Weerheijm et al., 2001). Clinically the enamel affected with MIH can vary in colour ranged from white to yellow / brownish opacities (Da Costa-Silva et al., 2011). Although MIH can affect multiple teeth, it is neither chronological in expression such as tetracycline staining or linear enamel hypoplasia, nor does it affect the entire dentition as seen in AI.

A classification to describe the severity of MIH has been suggested

1. Mild – lesion with only local colour change (white / opaque, yellow or brown) with smooth enamel surface
2. Moderate – lesion with rough and broken enamel
3. Severe – lesion affecting both enamel and dentine, atypical restorations replacing affected hard tissue

(Leppaniemi et al., 2001).

There is also an association between the enamel colour and increased severity of MIH, with the darker enamel opacities (brown and yellow) demonstrating a higher risk for PEB in the molar teeth (Da Costa-Silva et al., 2011). This study also showed a linear relationship between dental caries activity and severity of MIH.

The clinical problems related with MIH include aesthetics, rapid wear and enamel loss, increase susceptibility to caries, sensitivity to cold air, warm water, food and problems with tooth brushing which leads to poor oral hygiene and lastly tooth loss (Lygidakis, 2010).

2.6.5 Diagnosis

Criteria for diagnosis of MIH include presence of demarcated opacities, PEB, atypical restoration, extraction of molars due to MIH and failure of eruption of molar or an incisor. Examinations for MIH should be performed on wet teeth after cleaning. The most appropriate time to examine the teeth is at the age of 8 where all of the four permanent first molar and eight incisors have fully erupted. Table 2-1 shows the definition of the judgement criteria for MIH (Weerheijm et al., 2003).

Demarcated Opacities A demarcated defect involving and alteration in the translucency of the enamel, variable in degree. The defective enamel is of normal thickness with a smooth surface and can be white, yellow or brown in colour.
Post Eruptive Breakdown (PEB) A defect that indicate deficiency of the surface after eruption of the tooth. Loss of initially formed surface enamel after tooth eruption. The loss is often associated with pre-existing demarcated opacity.
Atypical Restoration The size and shape of restorations are not conforming the temporary caries picture. In most cases in molars it will handle about restorations extended to the buccal or palatinal smooth surface. At the border of the restorations frequently an opacity can be noticed. In incisor, a buccal restoration can be noticed not related to trauma.
Extracted molars due to MIH Absence of first permanent molar should be related to the other teeth of the dentition. Suspected extraction due to MIH are; opacities or atypical restorations in the other first permanent molars and the absence of first permanent molars in sound dentition in combination with demarcated opacities on the incisors is suspected for MIH. It is not likely that incisors will be extracted due to MIH.
Unerupted The first permanent molars or the incisors are not yet erupted to be examined.

Table 2-1 Definition of the judgement criteria for diagnosing MIH

2.6.6 Treatment and Management

Managing teeth affected with MIH is quite challenging due to the sensitivity and rapid development of caries, limited cooperation of a young child, difficulty in achieving anaesthesia and repeated marginal breakdown of restorations (William et al., 2006). Hypersensitivity in MIH teeth can lead to difficulty in oral hygiene care and further compromising the defective teeth. If the first permanent molar erupts with sign of enamel opacities and / or PEB and primary second molars are hypoplastic, the patient should be monitored in a regular basis until all of the first permanent molars have completely erupted.

As the PFM and the incisors are usually affected by MIH, therefore the child may receive dental treatment as early as six years of age. William et al. have suggested 6 steps in managing MIH in first permanent molar teeth as shown in Table 2-2 (William et al., 2006).

Restoring the PFM is not just selecting a suitable restorative material, but also involves managing children behaviour and determining how much of the affected enamel should be removed before the placement of the restoration material. Two approaches that have been described in determining cavity margin placement; removal of all defective enamel and only porous enamel is removed. The first approach can avoid premature failure of the restoration. However, this approach sacrifices the tooth structure. The latter approach is more conservative but there is risk of marginal breakdown (William et al., 2006).

In mild cases of MIH, fissure sealant is the treatment of choice where the enamel is still in good quality and caries free. Regular monitoring is essential, as PEB remains a risk and if it occurs, composite restoration can be placed in an appropriate time. In moderate cases, composite restoration is the treatment of choice ideally under rubber dam isolation. In severe form of MIH, the treatment options are either extraction or restoration. Decision to extract the affected molars depends on several factors such as occlusion, presence or absence of crowding, overall dental development, missing or malformed teeth and long-term prognosis. In cases where restoration is needed, a full coverage crown (stainless steel crown) is the treatment of choice (Daly and Waldron, 2009).

Steps	Recommended Procedures
Risk Identification	Assess medical history for putative etiological factors
Early diagnosis	Examine at risk molars on radiographs if available Monitor these teeth during eruption
Remineralisation and desensitisation	Apply localized topical fluoride
Prevention of dental caries and PEB	Institute thorough oral hygiene home care program Reduce cariogenicity and erosivity of diet Place pit and fissure sealants
Restorations or extractions	Place intracoronal (composite resin) bonded with a self-etching primer adhesive or extra coronal restorations (stainless steel crowns) Consider orthodontic outcomes post-extraction
Maintenance	Monitor margins of restorations for PEB Consider full coronal coverage restorations in the long term

Table 2-2 Clinical management for PFM affected with MIH (William et al., 2006)

Incisors affected with MIH may result in aesthetic concerns for the child and parents. Managing the incisors varies depends on the severity of the lesion, shallow creamy or whitish opacities may respond to simple micro abrasion, while yellow or yellow brown lesions can be bleached with carbamide peroxide (Lygidakis, 2010). However, bleaching with 10% – 38% carbamide peroxide is not recommended in immature teeth as it is frequently followed by side effects such as sensitivity, mucosal irritation and enamel surface alteration (Joiner, 2006).

Restorations with composite resin are the other alternative choice for incisors in children and adolescents with larger enamel defects (Wray et al., 2001). The larger size of the immature pulp chamber and pulp horns, and the immature gingival contour of the adolescent patient contra-indicates the use of porcelain veneers and therefore a conservative approach is required.

Another way that enamel formation can be disturbed is by inherited or genetic conditions.

2.7 Amelogenesis Imperfecta

2.7.1 Definition

"Amelogenesis imperfecta (AI) is a genetic condition, affecting the structure and clinical appearance of the enamel of all or nearly all the teeth in a more or less equal manner, and which may be associated with morphologic or biological changes elsewhere in the body". The inheritance can be autosomal dominant, recessive or X-linked (Aldred et al., 2003).

2.7.2 Epidemiology

The prevalence varies from 1:700 in Sweden to 1:14,000 in the USA. According to a review, there have been several classification systems applied to AI since 1945 and the latest was in 2003 (Crawford et al., 2007).

2.7.3 Classification

Originally, the classifications were based on phenotype such as hypoplastic, hypocalcification, hypomaturation, pigmented maturation and local hypoplasia (Witkop, 1957). A summary of the classification system for AI is shown in Table 2-3.

Weinmann et al. 1945	Based on phenotype: *hypoplastic and hypocalcified*
Darling, 1956	5 phenotypes based on clinical, radiograph and histopathology
	Hypoplastic
	Group 1 – generalised pitting
	Group 2 – vertical grooves (now known to be X-linked AI)
	Group 3 – Generalised hypoplasia
	Hypocalcified
	Type 4A – chalky, yellow, brown enamel
	Type 4B – marked enamel discoloration & softness with post-eruptive loss of enamel
	Type 5 – generalised or localised discolouration and chipping of enamel
Witkop, 1957	Based on phenotype. 5 types:
Hypoplastic	
Hypocalcification	
Hypomaturation	
Pigmented hypomaturation	
Local hypoplasia	
Schulze, 1970	Classification based on phenotype and mode of inheritance
Witkop and Rao, 1971	Based on phenotype and mode of inheritance

Hypoplastic

AD hypoplastic-hypomaturation with taurodontism

AD smooth hypoplastic with eruption defect and resorption of teeth

AD rough hypoplastic

AD pitted hypoplastic

AD local hypoplastic

X-linked dominant rough hypoplastic

Hypocalcified

AD hypocalcified

Hypomaturation

X-linked recessive hypomaturation

AR pigmented hypomaturation

AD snow-capped teeth

White hypomature spots

Winter and Brook, 1975 Based on phenotype and mode of inheritance as secondary means of sub-classification

Hypoplasia

Type I AD thin and smooth hypoplasia with eruption defect and resorption of teeth

Type II AD thin and rough hypoplasia

Type III AD randomly pitted hypoplasia

Type IV AD localised hypoplasia

Type V X-linked dominant rough hypoplasia

Hypocalcification

AD hypocalcification

Hypomaturation

Type I X-linked recessive hypomaturation

Type II AR pigmented hypomaturation

Type III Snow-capped teeth

Hypomaturation-hypoplasia with taurodontism

Type I AD smooth hypomaturation with occasional hypoplastic pits and taurodontism

Type II AD smooth hypomaturation with thin hypoplasia and taurodontism

Witkop and Sauk, 1976 Based on phenotype and mode of inheritance

Sundell and Koch, 1985 Based on phenotype

Witkop, 1988 Based on phenotype and subdivided into 15 subtypes by phenotype and secondarily

by mode of inheritance

Type I Hypoplastic

Type IA Hypoplastic, pitted AD

	Type IB Hypoplastic, local AD
	Type IC Hypoplastic, local AR
	Type ID Hypoplastic, smooth AD
	Type IE Hypoplastic, smooth X-linked dominant
	Type IF Hypoplastic, rough AD
	Type IG Enamel agenesis, AR
	Type II Hypomaturation
	Type IIA Hypomaturation, pigmented AR
	Type IIB Hypomaturation, X-linked recessive
	Type IIC Hypomaturation, snow-capped teeth, X-linked
	Type IID Hypomaturation, snow-capped teeth AD
	Type IIIA AD
	Type IIIB AR
	Type IV Hypomaturation-hypoplastic with taurodontism
	Type IVA Hypomaturation-hypoplastic with taurodontism, AD
	Type IVB Hypoplastic-hypomaturationwith taurodontism, AD
Aldred and Crowford, 1995	Classification based on:
	Molecular defect
	Biochemical result
	Mode of inheritance
	Phenotype
Hart *et al*., 2002	Proposed a molecular defect sub-classification of the AMELX condition
	Genomic DNA sequence
	cDNA sequence
	Amino acid sequence
	Nucleotide and amino acid sequences
	AMELX mutation described to date
Aldred *et al*., 2003	Classification based on:
	Mode of inheritance
	Phenotype – Clinical and radiographic
	Molecular defect
	Biochemical result

Table 2-3 Classification system for AI (Crawford et al., 2007).

Apart from these classifications, there are diagnosis codes that have been developed to group and identify diseases, disorders, symptoms, human response patterns and medical signs, as well as to measure morbidity and mortality. The International Classification of Disease (ICD) for hereditary disturbance in tooth structure is ICD-10 K00.5 if not classified elsewhere.

The diagnosis for AI can be made by family history, pedigree plotting chart and detailed clinical observation. Radiological findings may also help during the diagnosis, with respect to the degree of enamel mineralisation and taurodontism.

2.7.4 Aetiology

It is known that the formation of enamel is controlled by the interaction of organic matrix molecules including the enamelin (ENAM; 4q21), amelogenin (AMELX; Xp22.3-p22.1), ameloblastin (AMBN; 4q21), tuftelin (TUFT1;1q21) amelotin (AMELOTIN 4q13) dentine sialophosphoprotein (DSPP; 4q21.3) and numbers of enzymes such as kallikrein 4(KLK4; 19q13.3) and matrix metalloproteinase 20 (MMP20; 11q22.3-q23) (Crawford et al., 2007). Mutation of these genes has been found to cause different types of AI.

2.7.5 Clinical Presentation

Clinically, AI shows variation in the clinical presentation depending on the type of AI. In this study, the main focus is to study the phenotypic characteristic of the AI teeth. The clinical diagnosis of AI can be aided by asking the patient four questions to distinguish AI from other enamel defect such as fluorosis (Patel et al., 2013).

1. Has anyone else in the family had anything like this?
2. Has there been anything in the patient's medical history which might have caused sufficient metabolic disturbance to effect enamel formation?
3. Are all the teeth affected in a similar manner?
4. Is there a chronological distribution to the appearance to the defect?

The classification used is based on phenotype and also mode of inheritance as described by Winter and Brook, 1975.

2.7.5.1 Hypoplastic AI

Hypoplastic AI arises when an insufficient quantity of enamel matrix is formed and therefore reducing the thickness of enamel layer (Cogulu et al., 2009). However, the enamel that is formed is well mineralised. This is thought to occur as some areas of the enamel organ lack of IEE cells, causing a lack of differentiation into ameloblasts (Pinkham et al., 2005). There are several types of hypoplastic enamel including smooth, pitted (Figure 2-3) and rough (Figure 2-4). In the rough hypoplastic type, the enamel exhibits thin, hard, and rough surface. Teeth appear smaller in size with no proximal contact and areas of very thin or non-existent enamel resulting in sensitivity. Anterior open bite has also been reported in 60% of cases (Rowley et al., 1982).

Figure 2-3 Hypoplastic type of AI. Note the pitted enamel surface.
Image courtesy of (Crawford et al., 2007)

Figure 2-4 Hypoplastic type of AI with rough surface enamel. The teeth appear small in size with no proximal contact.
Image courtesy of(Crawford et al., 2007)

2.7.5.2 Hypocalcified AI

Hypocalcified AI is due to a defect in the calcification stage of enamel formation, characterized by normal enamel thickness but qualitatively the matrix is poorly calcified (Pinkham et al., 2005). Clinically, the enamel is soft and fragile especially at the incisal region, and may be lost soon after eruption, leaving the underlying dentine exposed leading to an unaesthetic appearance (Figure 2-5). The enamel has a cheesy consistency and can be scraped away from the underlying dentine. Large masses of supragingival calculus become deposited on the teeth, and this is frequently associated with severe gingivitis or periodontitis (Winter and Brook, 1975). Radiographically, the enamel is less radiopaque or similar to the dentine (Figure 2-6).

Figure 2-5 Hypocalcified type of AI. The enamel appears rough and discoloured. Image courtesy of (Crawford et al., 2007)

Figure 2-6 Panoramic radiograph of hypocalcified type type of AI. The radiodensities of enamel and dentine were similar. Image courtesy of (Ranganath et al., 2010).

2.7.5.3 Hypomaturation AI

Hypomature AI (Figure 2-7) is characterised by enamel of normal thickness but a lower value of radiodensity and mineral content, due to a mineralisation defect in the maturation phase of amelogenesis (Pinkham et al., 2005). Clinically, the crowns are normal in size and shape, have a rougher, duller and less refractive surface than normal enamel, and are more brittle as they show a tendency to fracture or chip but do not appear to be more susceptible to caries formation (Kim et al., 2005). The radiodensity of the affected enamel is generally less but can still be distinguished from the underlying dentine on radiographs. Teeth with mutations in KLK4 appear to have a homogenous dark yellow hue, while teeth with MMP20 mutations have an irregular greyish brown discolouration and are more glossy (Kim et al., 2005).

Figure 2-7 Hypomature type of AI. The enamel has normal thickness but with white opacity on the surface which can be mistaken with fluorosis. Image courtesy of (Crawford et al., 2007).

2.7.5.4 Hypoplastic/hypomaturation AI with taurodontism

The enamel appears mottled with a yellow brown colour, and pitted surfaces (Pinkham et al., 2005). Taurodontism is a condition found in the molar teeth whereby the body of the tooth and pulp chamber is enlarged vertically at the expense of the roots causing the floor of the pulp and furcation of the tooth is moved apically down the root (Jafarzadeh et al., 2008).

2.7.6 AI in primary teeth

AI can occur in primary and permanent dentitions. The literature on AI usually consists of case report and there is a lack of systematic reporting with regards to the clinical and radiographic findings. Thus, it leads to difficulty to appreciate its manifestation, dento-facial anomalies and the resulting treatment load.

The clinical presentation in primary teeth can be similar to permanent dentition, although primary teeth are usually less affected. A study was carried out to investigate a five-generation family member with AI. They found the exfoliated primary teeth also presented with loss of enamel thickness, rough surface, reduced enamel dentine contrast and PEB (Gjorup et al., 2009).

A previous study regarding the structure and composition of primary enamel affected by local hypoplastic autosomal dominant AI resulting from an ENAM mutation, showed a 'glass-like' appearance of enamel rod with reduced antibody towards ENAM protein (Shore et al., 2010).

Another case reported on a 4 year-old child who presented with tooth hypersensitivity and was diagnosed to have AI. Oral rehabilitation of the primary molars with stainless steel crown and resin-filled celluloid for the incisors were carried out to eliminate the sensitivity and reestablishment of normal occlusion resulted in improvement of the eating habit (Souza-e-Silva et al., 2010).

2.7.7 Management and Treatment of AI

Treatment varies for each AI patient, depending on the clinical presentation and should consider the patient's age, concerns, and severity. The objectives of treatment are to enhance the aesthetic appearance of the affected teeth, to reduce any sensitivity, preserve tooth structure and optimise masticatory function (Cogulu et al., 2009). In order to achieve those objectives, a multidisciplinary approach involving the Paediatric Dentist, Orthodontist and Prosthodontist are required (Claman et al., 2003).

Management of AI patient is a lifelong management. Starting with young children, which should be under the responsibility of Paediatric Dentist to provide support and reassurance not only to the patient but also to the parents regarding proper oral hygiene care, dietary concern, preservation of tooth structure and aesthetic, prevent pain, pathology and early tooth loss.

Bouvier et al. described the phases of treatment as the temporary, followed by the transitory phase (Bouvier et al., 1996). The primary dentition can be protected using preformed metal crowns for the posterior teeth, and composite restoration or polycarbonate crowns for anterior teeth if required.

The eruption of permanent teeth can be a difficult period, as some patients present with hypersensitivity or very fragile teeth, which can compromise oral hygiene. Management of permanent teeth vary depending on age, patient motivation, periodontal condition, endodontic status, loss of tooth structure, severity of disorder, socioeconomic status and cooperation of the patient. Patel et al. has described the management for AI teeth in adult patient in a comprehensive way. The treatment includes oral hygiene, dietary advice, desensitisation and stabilisation; bleaching and micro abrasion; crown lengthening surgery; composite resin restoration either direct or indirect; porcelain veneers; metal onlay; crown; dentures and implants (Patel et al., 2013).

CHAPTER 3

AIM AND OBJECTIVES

3 AIM AND OBJECTIVES

3.1 Rational for Research

Based on the current literature with regards to the prevalence and clinical problems, this study is generated to acquire more knowledge about the phenotypic characteristics in MIH and AI.

3.2 Aim

The aim of this study is to study the phenotypic characterisation of enamel MIH and AI in primary and permanent teeth, and compare to normal enamel.

3.3 Objectives

1. To investigate the type of defect and tooth discolouration in normal enamel and MIH and AI teeth
2. To measure the hardness value of normal enamel in control teeth and compare to MIH and AI teeth using Wallace indenter.
3. To investigate the chemical variation in hydroxyapatite in MIH and AI teeth compared to normal enamel using Micro Raman spectroscopy.
4. To study the ultrastructure of MIH and AI teeth using SEM and compare to normal enamel.

CHAPTER 4
PATIENT RECRUITEMENT AND SELECTION

4 PATIENT RECRUITEMENT AND SELECTION

4.1 Study registration and ethical approval

The study was designed and conducted in the Department of Paediatric Dentistry of the Eastman Dental Hospital UCLH NHS Foundation Trust (EDH). The study obtained ethical approval from the National Health Services Research Ethics Committee (NHS REC) in August 2011, registered under reference number 11/LO/0777. Project ID: 11/0223.

4.2 Patient Selection

Patients attending the Department of Paediatric Dentistry were approached and invited to participate in this study. In this study, the patients were divided into 3 groups; control, MIH and AI group. The inclusion criteria for patients in the control group were those who were fit and well, without any known illness or syndromes. Meanwhile, the exclusion criteria for the control group were patients with any known relevant medical illnesses, patients with deep caries lesions or any other dental anomaly and patients who did not understand English sufficiently to consent for the study. Control patients gave informed consent for the use of their teeth to be included in this study by signing the patient/parent consent form (Appendix 3 and 4), after a full explanation was given. The teeth were extracted as part of their dental treatment, such as orthodontic treatment.

Patient with enamel defects (MIH and AI) were recruited from the department or from the anomalies clinic, which runs once a month. Patients and parents who agreed to participate in the study were given a verbal explanation about the study and an information sheet (Appendix 1 and 2). Patients and parents who were happy to take part in the research gave written consent.

Every participant signed three copies of consent. The original copy of each form was kept in a filing cabinet in the locked office of the secondary supervisor, one copy was filed in the patient's clinical folder, and the last copy was given to the patient / parent for his / her reference.

4.3 Sample collection and storage

The samples were obtained and stored in accordance with the Human Tissues Act 2003. All of the control samples collected from the patients was either self exfoliated or extracted as part of the treatment plan.

In this study primary and permanent molar teeth were included as control samples as AI also can affect the primary teeth. It would be better if all 3 groups have the same number of teeth (primary and permanent). However, it was quite difficult to collect AI teeth due to the rarity of the condition, and only few of them required extractions. Therefore, both primary and permanent AI teeth were included in this study.

After extraction, the teeth were cleaned under running water to remove any blood or debris. Any intact gingival tissue or periodontal ligament was removed manually using tweezers or a scalpel blade. Teeth were then stored in 70% ethanol for one week to disinfect and to limit any possible further contamination. Teeth were then stored in thymol 0.1% for another one to four weeks before they were used. Each extracted tooth was stored in 0.1% thymol in an individual container, and placed in the refrigerator, at 4° Celsius as per department policy.

Each tooth sample was given a unique ID code, so that samples could not be identified as belonging to any individual patient, thus satisfying the requirement of good clinical practice, ethic and also patient's right of confidentiality.

CHAPTER 5
DEVELOPMENTAL DEFECT OF ENAMEL INDEX

5 DEVELOPMENTAL DEFECT OF ENAMEL INDEX (DDE INDEX)

Developmental defect of enamel can be either quantitative or qualitative in nature and can present with a range of clinical appearance. Over the past decades, various indices have been proposed for measuring enamel defect including fluorosis. These indices can be divided into 2 main categories: specific fluorosis indices and descriptive indices which comprising all types of defect (Clarkson and O'Mullane, 1989).

The most common indices to describe enamel fluorosis are Dean's Index, Tooth Surface Index of fluorosis and Thylstrup and Fejerskov fluorosis index. However, due to the difficulty to distinguish between fluoride and non-fluoride enamel defects and also the confusion in the classification, this lead to the development of a second group of indices, which included all types of enamel defects. However, these indices were descriptive in nature, causing further confusion.

In order to overcome these drawbacks, a Working Group of the World Dental Federation Commission on Oral Health Research and Epidemiology was established in 1977. The group proposed the used of a descriptive index entitle The Developmental Defect of Enamel (DDE) Index. This new index describes the enamel defect in term of type (opacity, hypoplasia, discolouration), number (single and multiple), demarcation (demarcated and diffuse), and location of defect (FDI 1982).

In this study, Dental Anomalies Proforma (Appendix 6) was used to record dental defect of enamel and / or dentine for each affected tooth. A modified version of the DDE index is included in the proforma. The DDE index is used to ensure the phenotype data for each tooth is recorded in an objective and systematic way. In this study the DDE index used is shown in Figure 5-1.

Apart from the DDE index, the proforma also comprises patient's demographic data, medical and dental history, ethnicity, diagnosis and family tree for relevant inherited enamel disease. Complete dental charting was also recorded and a separate section to record any enamel or dentine abnormalities and also whether any photograph or saliva sample has been taken. Radiographic findings were also recorded in this form. Finally, a complete treatment plan and management for the patient was written.

5.1 Methodology

All clinicians involved in using the Dental Anomalies Proforma were calibrated in a training session, showing clear photographs different types of enamel or dentine defect. The assessments were repeated after a month interval to determine reproducibility. At the end of the fifth training, 85% calibration was achieved involving 9 clinicians.

The DDE index comprises outer and inner boxes, which represent the permanent dentition and the primary dentition respectively. Any tooth presenting with an enamel defect is recorded in a particular box. The DDE index describes the enamel defect by location (L), demarcation (D), extension (E) and type (T) of defect and also records the severity of enamel wear (W) as shown below:

- Location (L):
  1 = incisal ½; 2 = gingival ½; 3 = whole surface
- Demarcation of defect (D):
  1 = demarcated; 2 = diffuse; 3 = both
- Extend of defect (E)
  1 = <1/3; 2 = 1/3 - 2/3; 3 = at least 2/3
- Wear (W)
  Mild; Severe

Enamel:

18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
DDE index: **Location (L)**: **1** incisal 1/2; **2** gingival 1/2; **3** whole surface. **Demarcation of defect (D)**: **1** demarcated; **2** diffuse;**3** both			55	54	53	52	51	61	62	63	64	65	**Extend of defect (E):** **1** <1/3; **2** 1/3 - 2/3; **3** at least 2/3. **Wear**: **mild** mild; **sev** severe		
			85	84	83	82	81	71	72	73	74	75			
48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38
Type of defect: **0** normal; **1** opacity (white/cream); **2** opacity (yellow/brown); **3** hypoplasia (pits); **4** hypoplasia (horizontal grooves); **5** hypoplasia (vertical grooves); **6** hypoplasia (missing enamel); **7** discoloured enamel (not assoc. with opacity); **8** post-eruptive breakdown; **9** other defects.															

Figure 5-1 DDE Index to record any type of developmental enamel defect for primary and permanent dentition.

Example of on how to record the enamel defect is as shown below according to the Figure 5-2.

Figure 5-2 Example of AI tooth affecting the permanent upper left second premolar

For the UL5 in the DDE index is:

L : 3 – whole surface

D: 2 – diffuse demarcation

E: 3 – defect affect more than two-third of the crown

W: severe

T: 6 – hypoplastic (missing enamel)

5.2 Results

5.2.1 Demographic data for collected tooth sample

The demographic details for each tooth sample were as shown below:

5.2.1.1 Control Sample

A total of 12 control teeth were collected from six patients, consisting of four second primary molars and eight first permanent molars. The demographic details of these patients are as shown in Table 5-1.

Patient ID	Age	Gender	Ethnicity	Type of tooth	Sample ID
33	15	Male	White	URE	C 93
				ULE	C 94
34	14	Female	White	UR6	C 95
				UL6	C 96
				LL6	C 97
				LR6	C 98
37	10	Female	Asian	URE	C 102
38	15	Female	White	UR6	C 106
				UL6	C 107
39	12	Male	White	UR6	C 108
				UL6	C 109
40	13	Female	White	URE	C 112

Table 5-1 Demographic details for control samples

5.2.1.2 MIH Sample

Twelve MIH teeth were collected from 3 patients with poor prognosis first permanent molars. These teeth were extracted as part of their treatment plan. The age ranged between 9 – 12 years old and the treatment were carried out either under inhalation sedation or general anaesthetic. The demographic details of the patients are as shown in Table 5-2.

Patient ID	Age	Gender	Ethnicity	Type of tooth	Sample ID
67	9	Female	White	UR6	MIH 33
				UL6	MIH 34
				LL6	MIH 35
				LR6	MIH 36
64	10	Male	White	UR6	MIH 32
				UL6	MIH 37
				LL6	MIH 38
				LR6	MIH 39
72	12	Male	White	UR6	MIH 49
				UL6	MIH 50
				LL6	MIH 51
				LR6	MIH 52

Table 5-2 Demographic details for MIH samples

5.2.1.3 AI Sample

Seven AI teeth were collected from three patients. A primary tooth, which self-exfoliated, two permanent teeth extracted due to poor prognosis, and four teeth extracted as part of orthodontic treatment were in the sample. The demographic details of the AI samples are as shown in Table 5-3.

Patient ID	Age	Gender	Ethnicity	Type of tooth	Sample ID
52	5	Male	White	ULA	AI 16
120	12	Female	Asian	UR4	AI 33
				LR4	AI 34
				UL4	AI 35
				LL3	AI 36
153	10	Male	Asian	UL5	AI 37
				LL4	AI 38

Table 5-3 Demographic details for AI samples

5.2.2 DDE Index

Pictures of the extracted MIH and AI teeth were taken by using a Canon EOS digital SLR camera with uniform flash for future reference. The DDE index was used to obtain the phenotypic information of each tooth.

5.2.2.1 MIH Samples

All of the defects in the MIH teeth were located in the incisal half of the crown, and were well-demarcated, extending to less than one third of the crown. Six of the teeth have white/cream (T1) opacity, three with yellow/brown (T2) opacity and three with PEB (T8) as shown in Table 5-4. Figure 5-3 show examples of white / cream, yellow / brown opacity and PEB defect.

Tooth Sample	Location	Demarcation	Extend of Defect	Wear	Type of Defect
MIH 32	1	1	1		1
MIH 33	1	1	1		2
MIH 34	1	1	1		2
MIH 35	1	1	1		1
MIH 36	1	1	1		1
MIH 37	1	1	1		1
MIH 38	1	1	1	mild	8
MIH 39	1	1	1	mild	8
MIH 49	1	1	1		2
MIH 50	1	1	1	mild	8
MIH 51	1	1	1		1
MIH 52	1	1	1		1

Table 5-4 DDE index for MIH samples

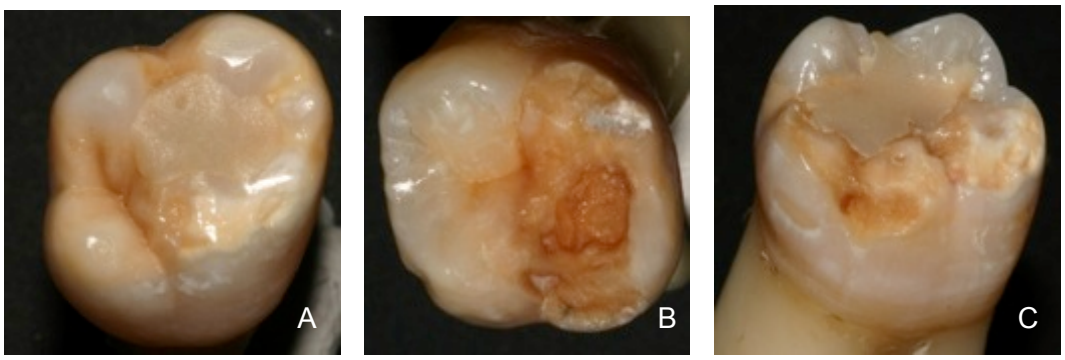

Figure 5-3 A; white/cream (T1) opacity for sample MIH 32, B; yellow/brown (T2) opacity for MIH 33 and C; post eruptive breakdown (T8) in MIH 39 with atypical restoration.

5.2.3 AI Samples

There was no enamel defects noted from the primary tooth collected. However, all of the permanent AI teeth showed severe enamel defects affecting the whole surface of the crown, with diffuse demarcation extending to at least two thirds of the crown. Teeth AI 33, 34, 35 and 36 were diagnosed as hypocalcified type of AI. The enamel defects affected the whole surface of the enamel and have yellow / brown (T2) type of defect. Teeth AI 37 and 38 were diagnosed as hypoplastic type of AI, with the enamel defect also affecting the whole surface of the enamel, AI 37 showed severe enamel wear, hypoplastic with deficient enamel, while AI 38 had yellow / brown (T2) discolouration (Figure 5-4). The description of the location and type defect as shown in Table 5-5. Tooth AI 2 showed yellow / brown type of defect, while the AI 3 was hypoplastic type of defect where the enamel layer is missing (T6).

Tooth Sample	Location	Demarcation	Extend of Defect	Wear	Type of Defect
AI 16					0
AI 33	3	2	3		2
AI 34	3	2	3		2
AI 35	3	2	3		2
AI 36	3	2	3		2
AI 37	3	2	3	severe	6
AI 38	3	2	3		2

Table 5-5 DDE index for AI teeth

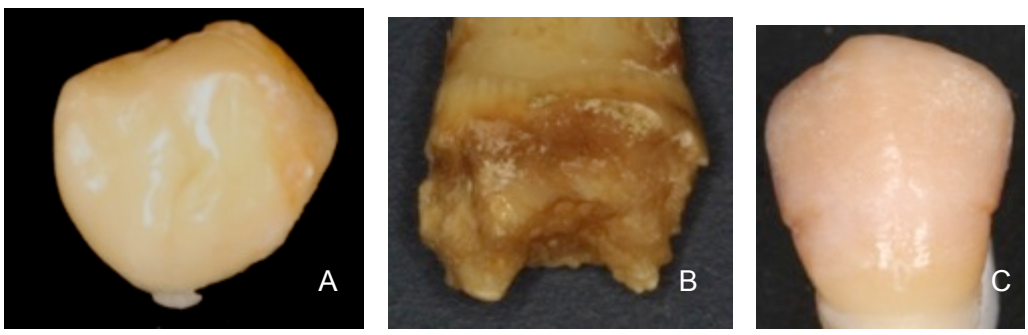

Figure 5-4 AI 16; (primary incisor) with no enamel defect (T0), B; AI 37 with missing enamel layer (T6), AI 34 with yellow / brown discolouration (T2).

5.3 Radiographic Findings

Periapical (PA) radiograph were taken for each extracted tooth, or any radiographic records available either dental panoramic tomography (DPT), upper anterior occlusal (UAO), bitewings (BW) or bimolar. Obvious radiographic features of the teeth with anomalies recorded included: pulp obliteration, shortened root, enlarged pulp space, bulbous crown, taurodontism and tooth wear. Information from the DDE form was transferred to the phenodent database.

5.3.1 Radiograph for control teeth

Radiographically, the control teeth show a clear difference in radiodensity between the enamel and dentinal layers separated by dentinoenamel junction (DEJ) as shown in Figure 5-5.

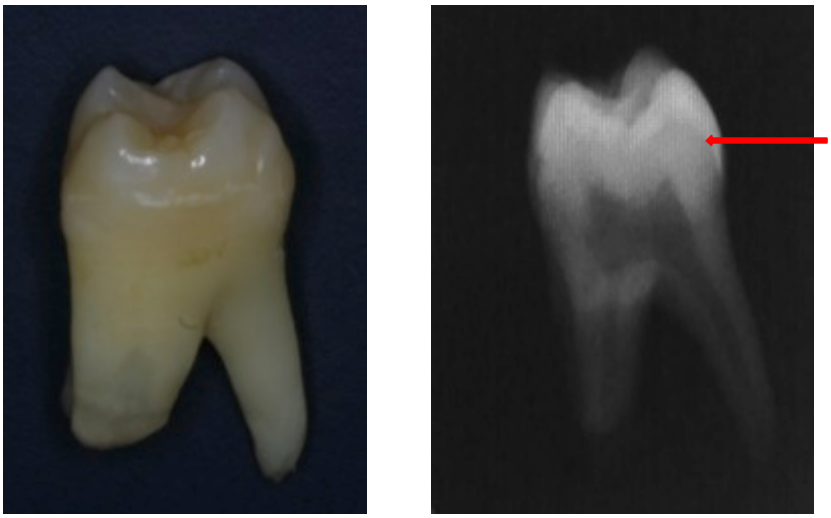

Figure 5-5 Periapical (PA) radiograph of control tooth (C 108). Arrow showed the DEJ.

5.3.2 Radiographic features for MIH teeth

The radiographic features for MIH were quite different from the AI teeth. The radiographic finding was dependent on the severity and size of the enamel defect and the depth of the lesion. In its very mild form of MIH (white / cream opacity), the lesion might not be visualised in the radiograph. However, in the severe form of MIH (yellow / brown (T2) or PEB, T8) the teeth present with radiolucency at the affected site, which resembled caries formation (Figure 5-6). The DEJ at the affected site was also diminished but could be clearly visualised on the unaffected site.

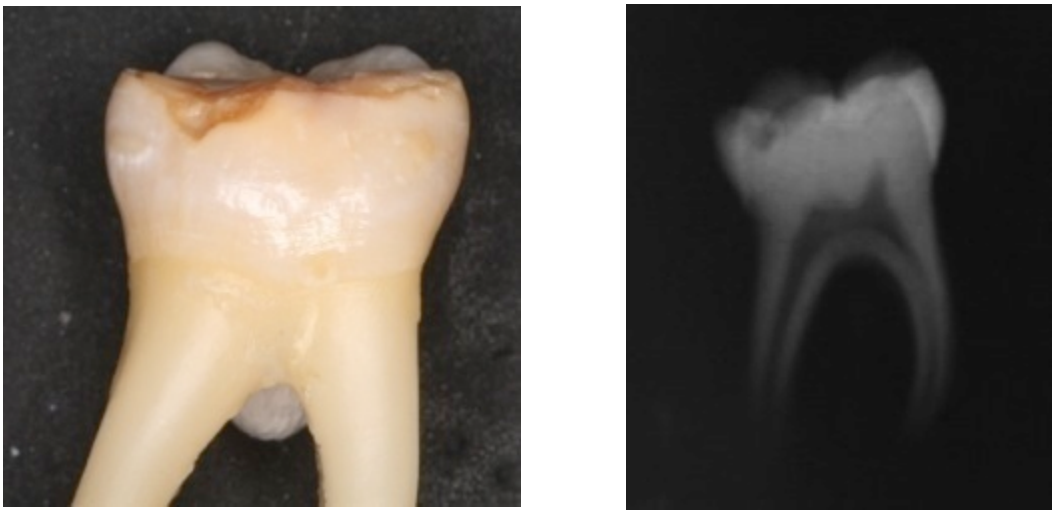

Figure 5-6 Periapical radiograph of MIH 39 shows radiolucency at the affected region. The DEJ clearly seen on the unaffected site different radiodensity between the enamel and dentinal layer.

5.3.3 Radiographic features for AI teeth

The radiograph of the permanent AI teeth showed no difference in radiodensity between the enamel and dentinal layers. Both layers appear the same and the DEJ is not clearly visualised (Figure 5-8). Some of the permanent molar teeth presented with taurodontism in the DPT (Figure 5-7 and 5-9).

Figure 5-7 A DPT of the patient for the teeth sample of AI 37 and AI 38 before the teeth were extracted (Hypoplastic type of AI). All of the first permanent molar showed enamel breakdown and the DEJ is not clearly identified. The UR6 and UL6 showed taurodontism of the pulp chamber.

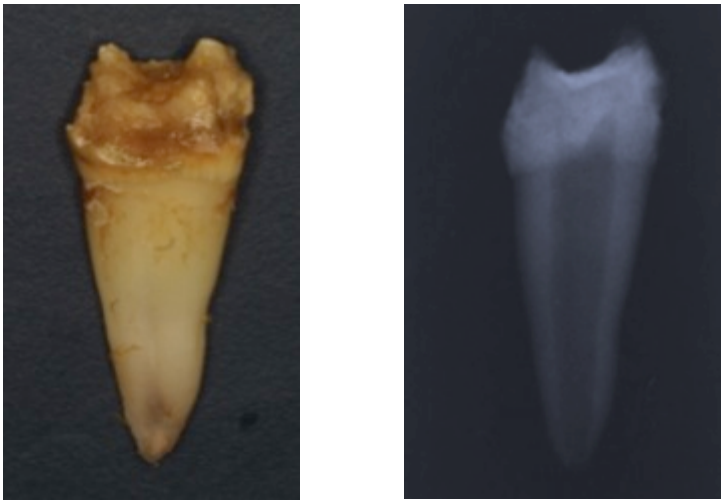

Figure 5-8 Periapical radiograph of tooth AI 37 after extraction which clearly showed diminished DEJ

Figure 5-9 A DPT of the patient for teeth sample AI 33, 34, 35 and 36 before the teeth were extracted (Hypocalcified type of AI). The UL7 and LL7 showed taurodontism.

5.4 Discussion

5.4.1 DDE Index

Various indices had been used to describe and measure enamel defects including fluorosis as described above. Most of the earliest indices were used to record fluorotic enamel. There are drawbacks with each proposed index, including insufficient information on the defect distribution in Dean's Index, and the criteria for diagnosis being limited to enamel defect making it difficult for the clinical examiner to distinguish between fluoride and non-fluoride origin in the Thylstrup and Fejerskov Index (Clarkson and O'Mullane, 1989).

In this study, the DDE index is used to describe the enamel defect because the it can describe the defect in a comprehensive way which include location, demarcation, extension, type and severity of defect. This index allows the clinician to record a broad range of enamel defects with no ascribing of aetiology. Filling up the form was quite time consuming especially if the defect affecting the whole dentition. However, all of the teeth sample in this study were recorded after the tooth being extracted and did not involve the presence of the patients.

The teeth in the MIH and AI groups showed marked discolouration compared to the control group. In the MIH group, the types of defect were white / cream opacity, yellow / brown opacity and PEB. Each defect represents the severity of the hypomineralised enamel in terms of the quality of the enamel. It has been shown that teeth presenting with yellow and brown opacity were at higher risk of developing PEB and atypical restoration than the white opacities (Da Costa-Silva et al., 2011). It could help the clinician to use the colour of the enamel opacity to anticipate future PEB and atypical restorations, to assist in assessing the long-term prognosis.

In the AI group, the teeth samples were from three patients with different type of AI. The primary tooth sample showed no enamel defect. Four of the teeth were diagnosed as hypocalcified type of AI, which show yellow brown opacity. Two teeth were from a patient with hypoplastic type of AI. One of the teeth has hypoplasia (missing enamel). However, the other tooth presented with yellow/brown opacities. Describing the defect of the enamel in AI teeth using the DDE index could be confusing as AI has different type of defects; hypoplasia, hypocalcification and hypomaturation. Extended Enamel Defect Index (EDI) and standardised image acquisition has been shown to provide

additional information with regards to the phenotypic description and quantification for enamel defect in AI (Smith et al., 2009).

Based on the DDE index, the enamel defect in MIH and AI teeth exhibit a particular characteristic that can be identified clinically. The MIH teeth presented with a well-demarcated lesion always affected the incisal/occlusal part of the crown. However, both hypoplastic and hypocalcified AI showed diffuse enamel lesion and affecting the whole surface of the crown. The differences between MIH and AI may aid the dentist when making a diagnosis. Clinically, the presentation of enamel defect in AI might be confused with fluorosis. Therefore, it is important to take a complete history mainly the family history and the amount of fluoride consumption in order to differentiate between AI and fluorosis.

From this study, DDE index is a useful tool to describe the phenotypic presentation in MIH and AI teeth although the colour description is limited to white/cream or yellow/brown opacity only. Although the defect was recorded extraorally, it is important to have an appropriate light source, which might conceal the defects. The teeth sample should also be well hydrated during the examination as a dehydrated sample can affect the presentation of the defect, as recommended by Clarkson and O'Mullane (Clarkson and O'Mullane, 1989).

5.4.2 Radiograph

The radiographs for control teeth revealed the different radiodensity between the enamel and dentine, as the enamel layer appeared more radiopaque than the dentine layer due to the different mineral content. The DEJ separates the enamel and dentine and was clearly identified in normal control teeth.

The radiographs for the MIH teeth show radiolucency at the region of the lesion similar to the radiographic presentation of caries. Radiographs play an important role in diagnosing the extension and perhaps the severity of the lesion. The PEB type of defects show clear radiolucency up to the dentine region. However, the white/cream and yellow/brown type of defects are less clear. However, diagnosis of MIH is not just dependent on radiograph findings. The judgement criteria that should be recorded in MIH includes: demarcated opacity, PEB, atypical restoration, extraction of molars due to MIH and failure of eruption of molar or incisors (Weerheijm et al., 2003). However,

radiographs are helpful in assessing the depth of lesions, which may effect the management.

The DPT of the hypoplastic type of AI patient showed generalised enamel breakdown and the size of the permanent dentition appear smaller. This is in accordance to the fact that hypoplastic AI is due to the deficient in the quantity of the enamel matrix that are formed. The contrast between the enamel and dentine was not clear and DEJ was hardly identified. The molar teeth also demonstrated taurodontism where the pulp chamber enlarges vertically and the pulpal floor moved apically which is the common finding in patient with AI.

The DPT of the hypocalcified AI patient showed teeth of generally normal size but the lack of contrast between enamel and dentine was similar to hypoplastic AI.

CHAPTER 6
ENAMEL COLOUR

6 COLOUR

6.1 Background

Colour can be described according to the Munsell colour system based on three dimensions in terms of hue, value and chroma as shown in Figure 6-1. This is a popular system for visual determination of colour (Joiner, 2004). It was created by Professor Albert H. Munsell in the first decade of the 20th century.

Hue is defined as the distinct characteristic of colour that distinguishes red from yellow from blue. These hues are largely dependent on the dominant wavelength of light that is emitted or reflected from an object. In the Munsell system, there are five principal hues; red, yellow, green, blue and purple along with five intermediate hues halfway between each principal hue. These include yellow-red, green-yellow, blue-green, purple-blue and red-purple. This is what we usually mean when we ask, "what colour is that?" (Kuehni, 2002).

Chroma is the 'purity' of a colour with lower chroma being less pure. There is no white, black or grey in a colour that has high chroma. It describes the degree of colour saturation and the strength and intensity or vividness of a colour (Kuehni, 2002).

Value is use to describe the colour as 'light' or 'dark'. In the Munsell system, black is Value 0 at the bottom and white is Value 10 at the top. Neutral greys lie along the vertical axis between black and white (Kuehni, 2002).

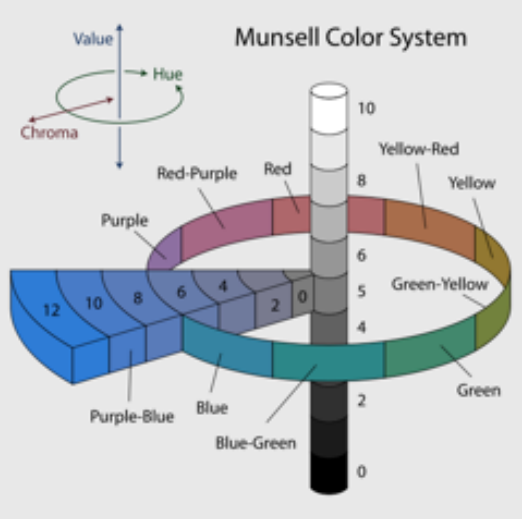

Figure 6-1 The Munsell Colour System. Measurement around the circle is the hue, the vertical pole represents the value and the horizontal away from the vertical pole is the measurement for chroma

6.1.1 Measurement of tooth colour

The colour and the appearance of teeth is a complex phenomenon. Factors that may influence the perception of tooth colour including the lighting conditions, translucency, opacity and light scattering, gloss and human perception (Joiner, 2004). Colour assessments may aid the dentist to determine the severity of the disease. A study was carried out to determine the relationship between colour and the severity in MIH patients, concluded that teeth with yellow and brown enamel opacities were more severely affected than white-cream (Da Costa-Silva et al., 2011).

Describing the colour of the enamel defect using the DDE index is just limited to white/cream and yellow/brown only. It is quite difficult to measure the colour of the defect solely by visual inspection especially inside the oral cavity because some of the lesion seems white/cream but actually yellow/brown type of lesion after being examined extraorally. This is might be due to proper light source when it is recorded after the tooth extracted.

Determination of colours can be carried out direct visual description, photographs or colorimetry that in this study is spectrophotometer. Direct visual description is highly subjective and influence by various factors including:(Franchi et al., 2009)

- Tendentiously, woman see colours better than men
- Young people perceive chromatic difference better than the older
- Tiredness
- Hydration of the teeth
- Colour fatigue
- Illumination

The variables in photographic image include the power of flash, film sensitivity, tilting of the frame and positioning the sample.

Methods to measure tooth colour include visual assessment via shade guides, spectrophotometry, colorimetry and computer analysis of digital images (Joiner, 2004).

Visual colour determination, by comparing the tooth with a standard colour tooth shade guides is the most commonly used method in dentistry (van der Burgt et al., 1985). However, although shade guides are frequently used as colour standards to which the colour of a tooth is matched, several drawbacks including inadequate range of available shades, the shades are not systematically distributed and lack of consistency

among and within individual dentists in matching the tooth colour (van der Burgt et al., 1990). Despite of these limitations, the use of shade guide is quick, easy and cost effective method for measuring tooth colour but lacks objectivity.

Spectrophotometry analysis measures one wavelength at a time from the reflectance or transmittance of an object. In dentistry, it has been used to measure the visible spectra of extracted and vital teeth (Joiner, 2004). This method had been demonstrated to be more accurate and more reproducible compared with visual shade assessment (83.3% compared to 26.6%) (Paul et al., 2002).

Colorimeter has colour filters that approximate the spectral function of the standard observer's eyes. In general colorimeters have shown good repeatability of natural tooth colour measurement either in vitro or in vivo (Joiner, 2004). However, the disadvantages of using colorimeters for measuring tooth colour includes the instruments are designed to measure flat surface, tooth surface is usually not flat and can have surface anomalies. The other drawback is the small aperture colorimeters are prone to significant edge-loss effect so colour determinations will be subject to errors (Joiner, 2004).

6.1.2 Spectrophotometric Shade Analysis

In this study, a spectrophotometer (SpectroShade™ Micro, MHT, Zürich, Switzerland) was used. The light output varies from 410nm to 680nm. This light is split so that teeth could be illuminated simultaneously from 2 sides at 45° angle. The reflected light was directed at 0° on the system's detector areas (~ 18 x 14 mm^2). One detector area is a colour CCD chip, which responsible to generate the coloured image and a black and white CCD detector area recorded the spectrophotometric data.

The spectrophotometer system uses a digital camera connected to a LED spectrophotometer. It can read the colour of a tooth and indicates the closest available chromatic standard. It also calculates the colour by using numerical difference between the natural tooth and the selected colour in terms of lightness/value (L), chroma (C) and hue (H) and compares them with those of the closest colour from a comprehensive library of shade standards.

The differences are expressed by a mathematical equation to calculate the Delta E (ΔE). The instrument software was used to compare the spectra from the tooth with the spectra from the colour library for selection of shade with minimal ΔE, according to the equation $\Delta E = \sqrt{[(L^*_{object} - L^*_{library})^2 + (C^*_{object} - C^*_{library})^2 + (H^*_{object} - H^*_{library})^2]}$ (Franchi et al., 2009).

ΔE is the overall indicator that specifies how close the selected shade is to the readings of the tooth based on value, chroma and hue. Generally, the lower the ΔE, the closer the match. For example, a ΔE value between 0 and 2 is considered to be excellent match, which can be described as the colour changes is undetectable to the human eye. ΔE between 2 and 4 are considered a good match and any ΔE above 4 is considered borderline, as the colour difference is detectable by human eye especially when the value (L) difference exceeds 2 (MHT, 2005).

The aim of using Spectroshade is to measure the colour and also to compare two similar colours and determine the difference. It can read and measures the lightness (Value) either dark to bright, intensity of the colour (Chroma) from weak to strong and the colour of the tooth (hue).

6.2 Methodology

The tooth colour measurements were recorded using the spectrophotometer (SpectroShade^{TM} Micro-Dental Spectrophotometer) (Figure 6-2). Before each shade measurement was taken the spectrophotometer device was calibrated using the White and Green Calibration Tiles (Figure 6-3). The extracted teeth were placed in a mouth simulator system for the shade measurements (Figure 6-4).

After calibration the device was positioned against the tooth to be measured. Camera window opened and automatically, displaying the yellow target box in the centre. Then, the ‘MEASURE’ button was pressed and the captured image displayed in ‘Today’s Reading’ window.

On completion of the spectrophotometer measurements the data were uploaded to a PC via USB and saved onto the MHT software.

Figure 6-2 Spectrophotometer

Figure 6-3 Calibration tiles

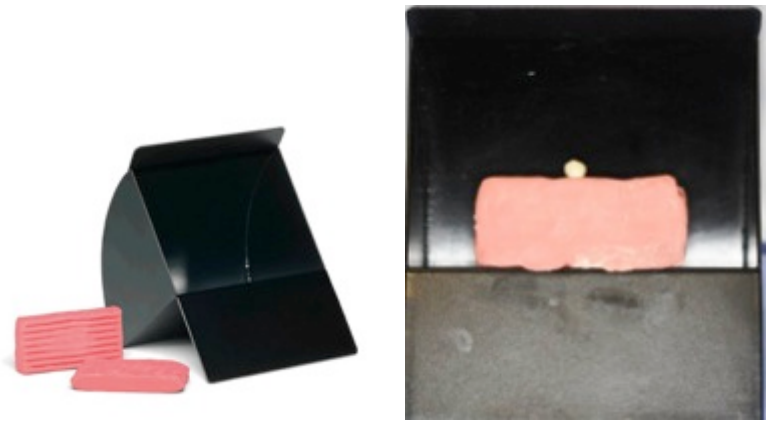

Figure 6-4 Mouth simulator to imitate oral cavity is used for the extracted tooth sample before the measurement carried out.

The spectrophotometer software allowed the L*C*H° values to be recorded for each samples. These values then allowed the calculation of the delta ΔE scores, which represents the overall change in colour difference that specifies how close the selected shade to the readings of the tooth based on an average of value, chroma and hue (LCH) as explained in section 6.1.

An example of the measurements taken by the spectrophotometer to determine the value for LCH to be obtained and calculate the ΔE for each tooth to obtain a quantifiable value between the control, MIH and AI group are as shown in Figure 6-7.

For control teeth, the L, C and H values were taken at the middle third of the crown to get the ΔE value (Figure 6-5). However, for MIH and AI group, the values were taken at the most affected region either on the occlusal surface or the other tooth surfaces (Figure 6-6). So, the spectrophotometer will measure the colour difference between the normal tooth surface and the most affected surface, which has became discoloured due to MIH or AI.

Figure 6-5 For control teeth, the L, C and H values were taken at the middle third of the crown of the tooth.

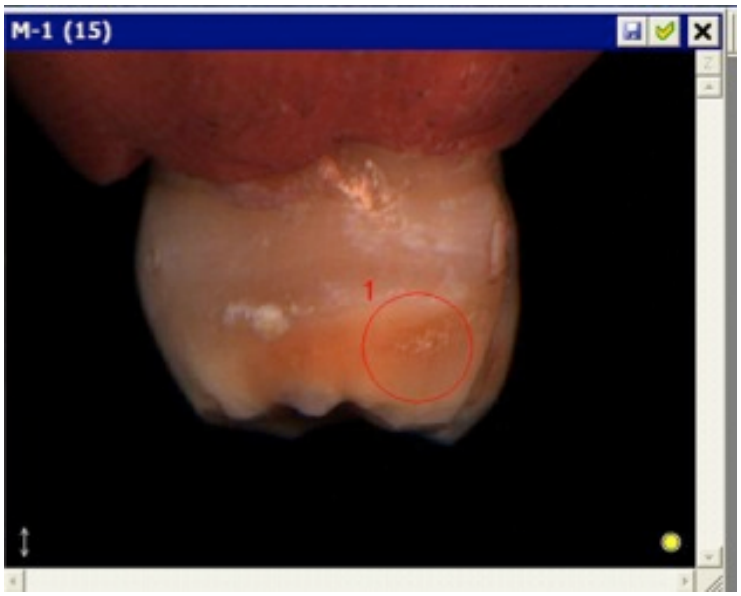

Figure 6-6 MIH tooth with enamel discolouration at the occlusal third of the crown. The L, C and H values were taken at the most affected region as shown in the red circle.

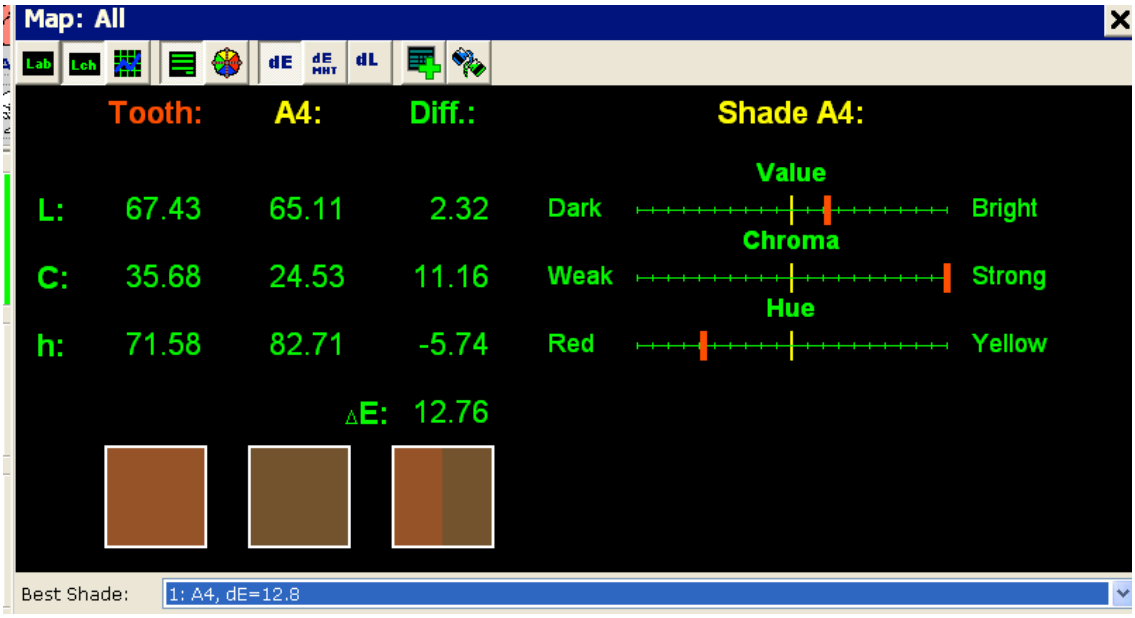

Figure 6-7 This screen provides the L, C and H values of the tooth, comparing with the library shades which is A4 and also the different values of L, C and h between the tooth and the library shades. At the bottom it also shows the ΔE that has been calculated (12.76). On the lower left corner, there are three colour boxes. The first box indicate the colour of the tooth sample, the second box is the colour indicator of the closest overall shade standard and the last box with split down in the middle showing the sample tooth shade on the left and the corresponding standard shade on the right.

Lightness (Value) represents the brightness of the colour. A dark tooth (Shade A4, C4) will have low value while a bright tooth (Shade A1, B1) will have higher value. The strength or saturation of the colour was measured in Chroma. Usually the incisal tends to be more translucent and this will produce a weak chroma value than a decalcified area on the same tooth. In Figure 6-7, the ΔE is 12.76, which represent the overall colour difference, which specifies how close the selected shade is with the reading of the tooth base on the average of Value, Chroma and hue.

6.3 Results

For the control group, the ΔE values were low, which was considered as an excellent or good match comparing to the available library shade except for tooth C94 and C95. This was may be due to the presence of carious lesions that can affect the colour of the enamel when the image was taken (Table 6-1).

The MIH group, showed more variation in ΔE values. The lowest value was 2.57 for the white/cream type of lesion (MIH 37) and the highest was 21.37 for MIH 50 (PEB). High ΔE values were also noted among the white/cream lesions (Table 6-2).

The primary AI (AI 16) showed an excellent match with the library shade, as there was no enamel defect noted (**ΔE=1.34)**. However, the rest of the teeth samples, which were permanent AI teeth had high ΔE value. The values ranged between 5.24 and 7.87 indicating that the colour changes were identifiable to human eye (Table 6-3).

The different shade column on the left side of each table represent the overall shade selection for the tooth comparing with the standard manufacturer shade guides.

Tooth Sample	ΔL	ΔC	ΔH	ΔE	Different Shade
C 93	0.53	0.61	1.22	1.46	D2
C 94	0.88	5.60	0.63	5.71	C1
C 95	0.54	4.81	3.44	5.93	D2
C 96	0.64	2.06	0.64	2.36	A3
C 97	1.01	1.58	0.13	1.88	A3
C 98	1.21	0.37	0.79	1.49	A2
C 102	0.43	1.43	2.29	2.73	A3.5
C 106	1.15	0.84	3.37	3.65	C1
C 107	0.23	0.67	1.36	1.53	D2
C 108	0.17	1.67	2.80	3.27	A3
C 109	0.96	0.86	1.39	1.90	B2
C 112	0.36	1.06	0.13	1.12	A2

Table 6-1 The ΔL, ΔC, ΔH and ΔE for control teeth. On the right side of the table also shows the closest shade of each tooth with the standard manufacturer shade guide.

Tooth Sample	Type of defect	ΔL	ΔC	ΔH	ΔE	Different Shade
MIH 32	White/cream	2.01	5.05	5.54	7.76	B4
MIH 33	Yellow/brown	17.62	13.75	9.98	24.48	C4
MIH 34	Yellow/brown	5.51	5.27	4.25	8.73	A4
MIH 35	White/cream	1.76	2.37	3.26	4.40	A4
MIH 36	White/cream	0.91	1.55	2.55	3.12	A3.5
MIH 37	White/cream	0.65	1.08	4.72	2.57	C4
MIH 38	PEB	0.98	3.93	4.72	6.22	A4
MIH 39	PEB	5.22	2.73	4.65	14.28	C4
MIH 49	Yellow/brown	2.05	0.74	2.28	3.82	C4
MIH 50	PEB	16.91	10.27	8.08	21.37	C4
MIH 51	White/cream	0.33	2.23	2.36	3.26	C3
MIH 52	White/cream	0.27	2.04	1.58	2.59	D2

Table 6-2 The ΔL, ΔC, ΔH and ΔE for MIH teeth. Comparing the ΔE and the type of defect for each tooth

Tooth sample	Type of Defect	ΔL	ΔC	ΔH	ΔE	Different Shade
AI 16	Normal	0.56	1.13	0.45	1.34	C1
Hypocalcified AI						
AI 33	Yellow/brown	0.58	0.69	5.16	5.24	D3
AI 34	Yellow/brown	0.92	0.15	7.15	7.21	C3
AI 35	Yellow/brown	1.05	0.37	6.03	6.13	D3
AI 36	Yellow/brown	0.23	1.74	7.38	7.58	C4
Hypoplastic AI						
AI 37	Hypoplastic (missing enamel)	7.55	0.20	2.23	7.87	C4
AI 38	Yellow-brown	1.53	4.16	3.25	5.50	B4

Table 6-3 The ΔL, ΔC, ΔH and ΔE for AI teeth. Comparing the ΔE and the type of defect for each tooth.

The summary of the ΔE values for control, MIH and AI teeth are shown in Figure 6-8. ΔE values of more than 4 indicate that the colour changes can be identified by human eyes, whereas those below were considered a good match and undetectable to the human eye.

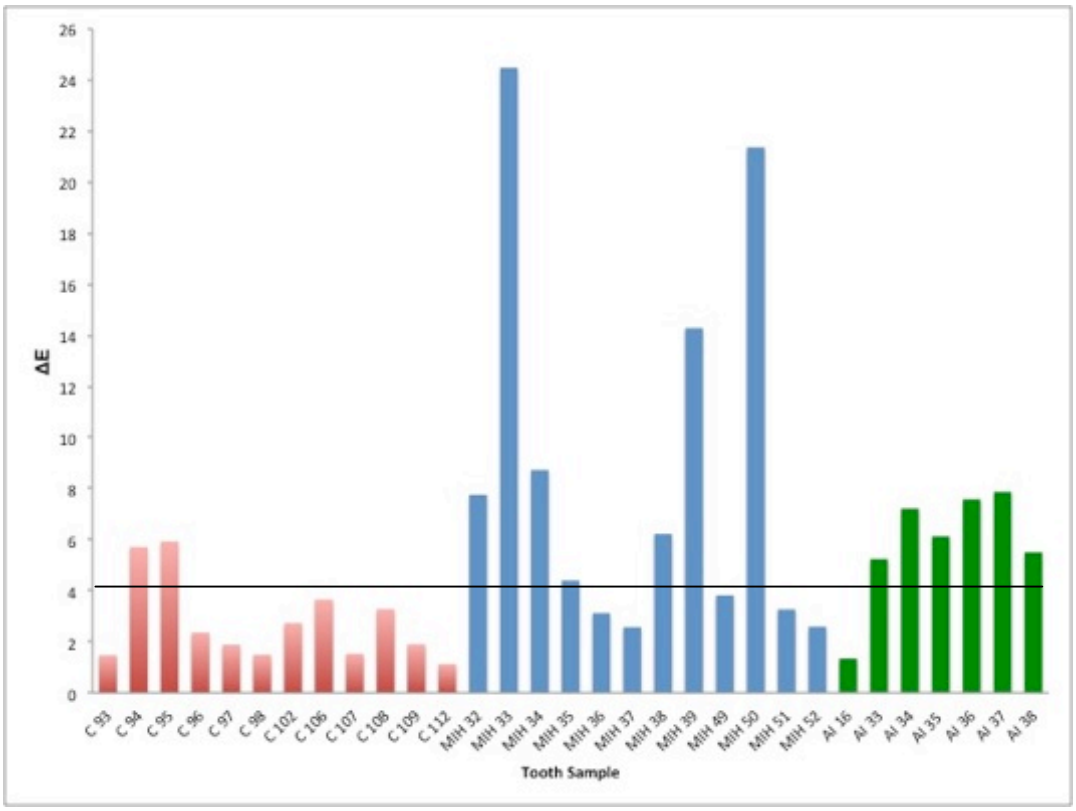

Figure 6-8 The summary of the ΔE comparing between the control, MIH and AI group. Red bars represent control teeth, blue bars represent MIH teeth and green bars indicate AI teeth. ΔE values more than 4 indicate the colour changes are considerably visible and noticeable by human eye.

6.4 Discussion

In this study, the samples either had no lesion (T0), white/cream (T1), yellow/brown (T2) or PEB (T8) type of lesions. It is difficult to describe the colour of the defective enamel if it is just base on the DDE index.

In order to improve the enamel colour measurement more objectively, the use of spectrophotometer analysis (MHT Spectro Shade) is more accurate and reproducible compared to human shade assessment (Paul et al., 2002). Spectrophotometer allows the measurement of the lightness (value), hue and chroma of the teeth and was then compared with those of the closest colour of the standard shade. Once the closest colour identified, the spectrophotometer will allow the calculation of the differences between the two colours as expressed in ΔE.

Spectrophotometers are usually used by dental practitioners to find the best colour match for the patient in the fabrication of porcelain crowns. In scientific research, spectrophotometer is used to measure tooth colour changes at several different time points, mainly when studying the effect of treatment, such as dental bleaching. However, in this study the comparison was between the tooth sample and the standard shade only and not based on different times.

However, the use of the DDE index was also helpful to assess the level to discolouration of the enamel defect. The degree of staining of hypomineralised enamel as assessed visually may be used to reflect the severity of the defect (Farah et al., 2010a).

The control samples showed lower values of ΔE as the distance of the colour changes was closer to the colour standard, except for two teeth where the ΔE value was more than 4, possibly due to the presence of caries that could affect the colour of the tooth. During the measurement, all of the tooth samples were prone to dehydration due to air-drying. So, the measurement was carried out as fast as possible to prevent dehydration that may affect the colour of the enamel.

There were variations of ΔE values in the MIH group depending on the different types of defect. The white/cream lesions appeared to have lower ΔE values, e.g. less than 4. While the yellow/brown and PEB lesion showed higher ΔE values. The PEB lesion had the highest ΔE values: up to 21 for tooth MIH 50. Though sample MIH 38 presented

with PEB lesion, the ΔE value was only 6.22. This may have been because most of the surface of the lesion was covered with filling material, which may have affected the spectrophotometer measurement.

In the AI group, the DDE index showed that the lesion was diffuse and involved almost the whole of the enamel surface. Clinically, the discolouration in hypocalcified AI appeared more homogenous than the hypoplastic AI as the presence of missing enamel may expose the underlying dentine. However, all of the permanent AI teeth showed ΔE values of more than 4, which were detectable to the human eye. In these patients, the enamel defect involved the whole dentition, leading to aesthetic concerns, which is one of the main complaints when patients attend the dental clinic. A study on patients with AI showed that patients had higher levels of social avoidance and distress (Coffield et al., 2005). Therefore it is important to identify the best shade to restore the teeth especially for the older children, so that they will have better quality of life and improve their social relationship.

In this study, the analysis for each parameter (light, chroma and hue) was not carried out because of the time limitation of the study. It would be worthy to analyse each component of the parameter to see which of the component is most affected in MIH and AI.

CHAPTER 7

ENAMEL HARDNESS

7 HARDNESS

7.1 Background

Hardness is the property of a material that enables it to resist plastic deformation, usually by penetration / indentation. However, the term hardness may also refer to resistance of the material to bending, scratching, abrasion or cutting. Hardness tests measure the resistance of the material to plastic or permanent deformation by a sharp object under constant load (Angker and Swain, 2006).

There are three type of hardness test; macro, micro and nanohardness depending on the load used. Macrohardness study refers to static indentation with load more than 1 kilogram-force (kgf). On the other hand, microhardness test is indentation with load of less than 1 kgf. In this study, the load test weighted 300 gf (2.94N), therefore it was categorised as a microhardness test study.

Microhardness tests are widely used to investigate the physical properties of teeth. In dentistry, the value for enamel hardness has been analysed either by using the Knoop Hardness Number (KHN) or by Vicker Hardness Number (VHN) depending on the type of test; Knoop indenter or Vickers indenter.

The Vickers Hardness use a square-based pyramid indenter whose opposite side meets at the apex at the angle of 136° (Figure 7-1). The indenter is pressed onto the surface of specimens and the size of impression is measured using the above formula.

The Vickers Hardness Number is determined by the ratio F/A where F is the force applied to the diamond, and A is the surface area resulting from the indentation, where d is the average length of the diagonal left by the indenter. This formula is derived from the geometry of the indenter and area of indentation (Figure 7-1).

Figure 7-1 Schematic diagram showing the shape of the Vickers indenter and impression. The Vickers hardness number is calculated based on the surface area of the indent (Adapted from Wallace indentation hardness tester instruction manual).

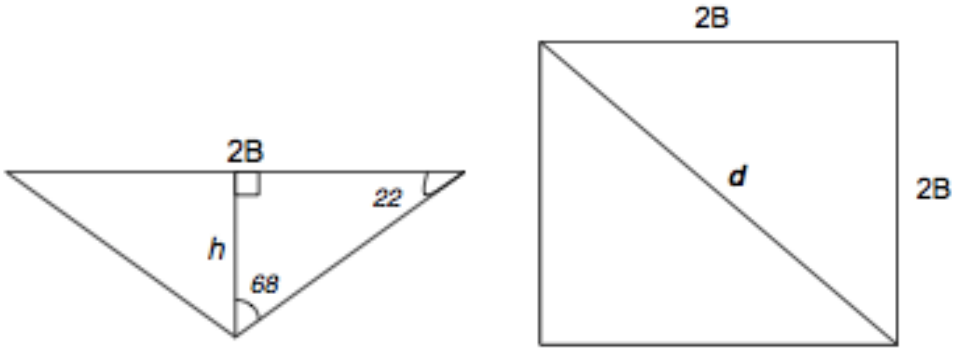

Figure 7-2 (Left) Cross section view of indenter and (Right) top view of indentation. h is the depth of indentation and d is the diagonal of indentation

Based on the geometry of the indenter (Figure 7-2), the area of indentation can be calculated from the following formula:

$$A = d^2 / 2 \sin (136°/2)$$

which can be approximated by evaluating the sin term to give

$$A \approx d^2 / 1.8544$$

where *d* is the average length of the diagonal left by the indenter in millimetres. Hence,

$$HV = F/A \approx 1.8544F/d^2$$

where *F* is in kgf and *d* is in millimetres.

The basic formula above was adapted in this study to measure the hardness of dentine as given below (Fuentes et al., 2003):

$$VHN = 1854.4F / d^2$$

where F is the load in grams and d is the mean diagonal of indentation in micrometres. Thus the unit used in this study is expressed as grams per micrometres squared.

Faria-e- Silva *et al.* showed that normal enamel hardness is significantly higher (KHN 360.4) than enamel affected by AI (KHN 53.3) using the Knoop hardness indenters (Faria-e-Silva et al., 2011). However, there is no significant different in dentine hardness between the normal enamel and enamel with AI. The study also demonstrated there is a positive linear relationship between hardness and bond strength for enamel. It was postulated that the higher mineral content of enamel is expected to generate a better mechanical interlocking with the adhesive resin therefore enhancing the enamel bond strengths. However, there was no significant relationship between hardness and bond strength for dentine. Most of the current data are demonstrating the enamel hardness with AI in permanent teeth.

7.2 Methodology

Tooth sample were examined using the Wallace indentation hardness tester (H.M Wallace, Croydon, England – serial number 067851/1) (Figure 7-3). One of the factors that can affect the hardness measurement is the difficulty to stabilize the tooth sample due to the convexity of the crown. Therefore, the cleaned tooth samples were mounted onto the sample plate of the Wallace indenter and stabilised using reusable putty-like pressure sensitive adhesive so that the tooth sample did not move during the measurement taken. The adhesive was applied surrounding the tooth and did not reach the bottom of the tooth to prevent false readings. The orientation of the indenter in relation with the enamel surface was perpendicular at 90 degrees and all measurement were taken with a micro-indentation using the Wallace indenter at load of 300g.

Figure 7-3 Wallace indenter as an instrument to measure the enamel hardness

For each of the teeth, fifteen measurements were obtained randomly and for the test group (MIH and AI) another fifteen measurements were taken on the affected surface. The load applied and held in place for 15 seconds. The depth of indentations was then recorded. For each sample, an average of hardness measurements were expressed as VHN.

7.2.1 Statistical Analysis

The statistical analysis for VHN values for enamel in control, MIH and AI group was carried out using SPSS Version 22. The hardness (VHN) data were analysed by Multilevel Model Analysis.

7.3 Result

7.3.1 Wallace Hardness for Control Teeth

A total of twelve control teeth were measured using Wallace indenter to obtain the VHN and fifteen readings were taken for each tooth sample. The average readings for each tooth are shown in Table 7-1. Figure 7-4 shows the mean enamel hardness for control group plotted as bar graph. The VHN values ranged from 222.35 to 360.32 VHN. The mean VHN for enamel in control teeth was 315.85 (± 61.69) VHN.

Tooth	d(diagonal) µm	VHN (1854.4*300/d^2)	Standard Deviation	GPa	Standard Deviation
C 93	39.90	356.64	61.98	3.50	0.61
C 94	50.13	226.35	42.71	2.22	0.42
C 95	40.66	341.59	48.27	3.35	0.47
C 96	50.80	222.64	50.81	2.18	0.50
C 97	42.50	313.85	50.14	3.09	0.49
C 98	42.33	316.47	51.73	3.10	0.51
C 102	41.50	326.03	36.71	3.20	0.36
C 106	42.33	312.55	30.52	3.07	0.30
C 107	41.83	321.57	39.45	3.15	0.39
C 108	39.48	360.32	43.32	3.53	0.42
C 109	40.49	343.21	42.91	3.37	0.42
C 112	43.42	299.04	41.65	2.93	0.41

Table 7-1 VHN values for control sample. Mean VHN value for control teeth is 311.69 (± 61.69).

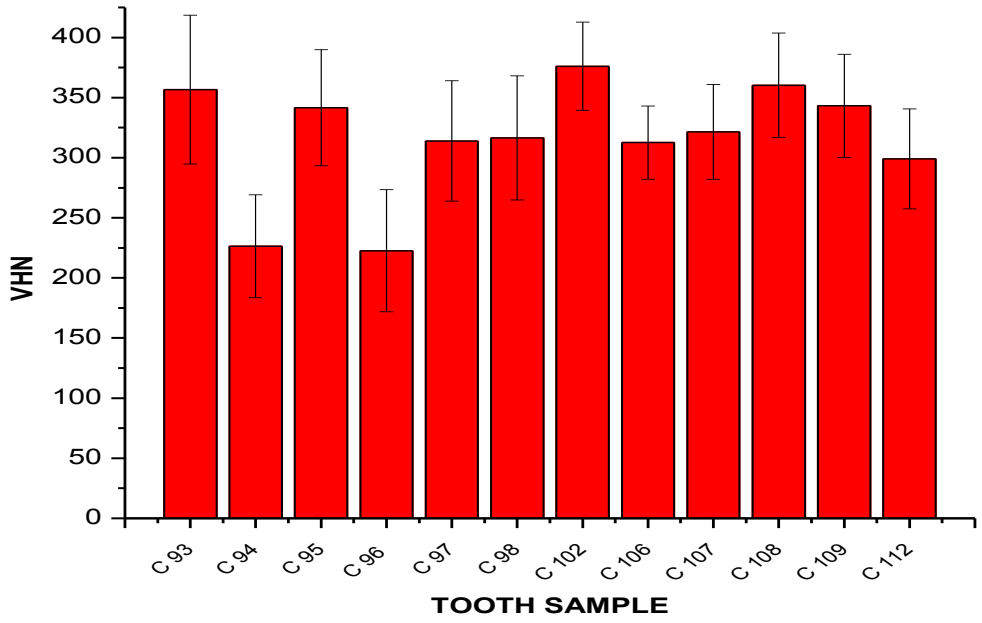

Figure 7-4 Mean enamel hardness (VHN) for 12 control teeth. The Y-axis represents the mean VHN and the X-axis represents the twelve control teeth. The error bar represents the standard deviation for each sample.

7.3.2 Wallace Hardness for MIH Group

In the MIH group, twelve MIH teeth were measured, 15 readings were taken separately on both the affected surface and on the normal enamel surface. Table 7-2 shows the enamel hardnes in MIH teeth on the affected site, which are also comparison by type of defect. The VHN values for the PEB (MIH 38, 39 and 50) type of lesion showed the lowest values, which are 42.97, 37.83 and 44.11 respectively. The white/cream lesion hardness ranged between 76.96 minimum and 105.90 maximum, while the yellow/brown defect ranged between 51.42 and 63.91.

Figure 7-5 shows the comparison between the hardness on normal enamel surface (blue bar) and on the affected site (red bar). The VHN for the unaffected surfaces was within the same range as the control group. However, the VHN for the affected surface showed a marked reduction in enamel hardness compared to the control group and compared to the unaffected sites.

Tooth	Type of defect	d(diagonal) µm	VHN ($1854.4*300/d^2$)	SD	GPa	SD
MIH 32	White/cream	80.31	86.79	8.14	0.85	0.08
MIH 33	Yellow/brown	93.55	63.91	5.69	0.63	0.06
MIH 34	Yellow/brown	104.11	51.42	2.76	0.50	0.03
MIH 35	White/cream	82.40	82.49	8.32	0.81	0.08
MIH 36	White/cream	82.32	82.58	7.64	0.81	0.07
MIH 37	White/cream	82.57	81.92	6.15	0.80	0.06
MIH 38	PEB	114.01	42.97	3.20	0.42	0.03
MIH 39	PEB	121.38	37.83	1.98	0.37	0.02
MIH 49	Yellow/brown	100.01	56.43	8.14	0.55	0.08
MIH 50	PEB	112.58	44.11	3.69	0.43	0.04
MIH 51	White/cream	85.25	76.96	6.86	0.75	0.07
MIH 52	White/cream	73.18	105.90	18.35	1.04	0.18

Table 7-2 VHN values for MIH teeth on the affected site comparing with type of defect

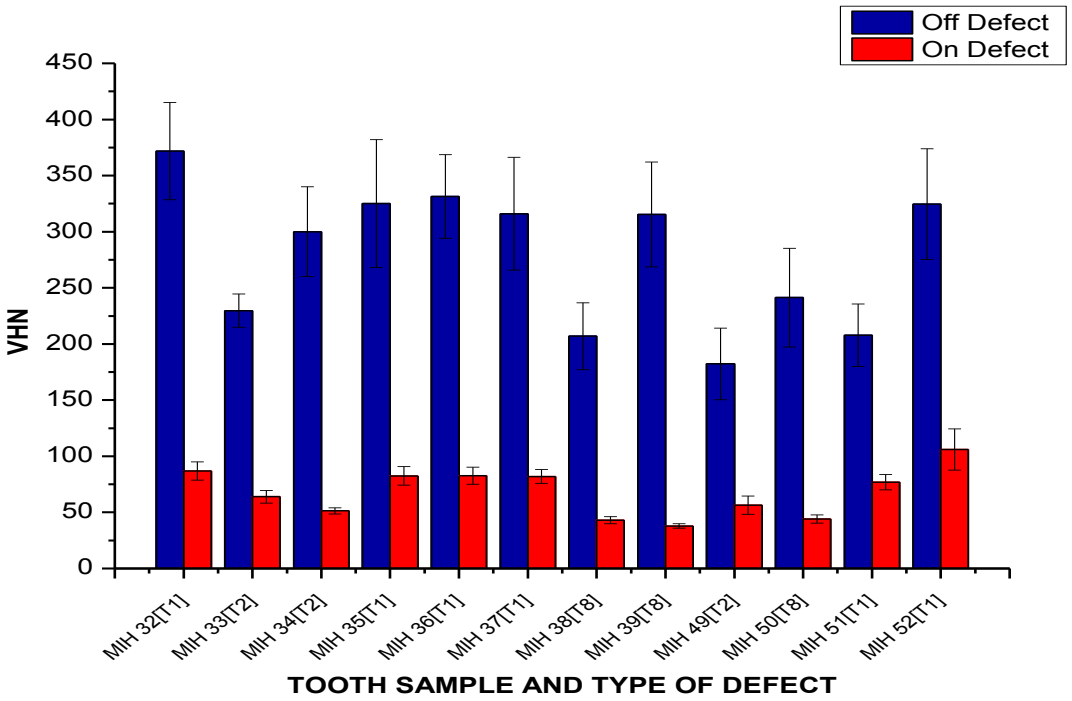

Figure 7-5 Mean enamel hardness (VHN) for MIH teeth. Blue bar shows the VHN on the normal surface and red bar for the affected surface. The error bars indicate the standard deviation for each tooth.

7.3.3 Wallace Hardness for AI Group

In the AI group, seven AI teeth were measured, including one primary tooth and six permanent teeth. Fifteen measurements were taken for each tooth. Table 7-3 and Figure 7-6 show the mean enamel hardness in AI group associated with the type of defect.

The enamel hardness for the primary tooth (AI 16) is within normal enamel hardness ranged of 242.58 (± 65.76). The permanent teeth with AI showed great reduction in enamel hardness compared to the control teeth, especially the tooth with hypoplastic (missing enamel) type of defect where the hardness was very low (27.33 ± 2.34). The teeth AI 33, 34, 35 and 36, which are from a patient with hypocalcified AI revealed a constant hardness values between 100.40 (±11.24) to 117.29 (± 10.46).

Tooth	Type of defect	d(diagonal) µm	VHN ($1854.4*300/d^2$)	SD	GPa	SD
AI 16	Normal	49.21	242.58	65.76	2.38	0.64
Hypocalcified AI						
AI 33	Yellow/brown	72.76	105.55	8.36	1.04	0.08
AI 34	Yellow/brown	69.07	117.29	10.46	1.15	0.10
AI 35	Yellow/brown	74.77	100.40	11.24	0.98	0.11
AI 36	Yellow/brown	69.24	116.91	12.26	1.15	0.12
Hypoplastic AI						
AI 37	Hypoplastic (missing enamel)	143.01	27.33	2.34	0.27	0.02
AI 38	Yellow-brown	74.86	100.88	15.56	0.99	0.15

Table 7-3 Mean enamel hardness (VHN) for AI teeth comparing with type of defect

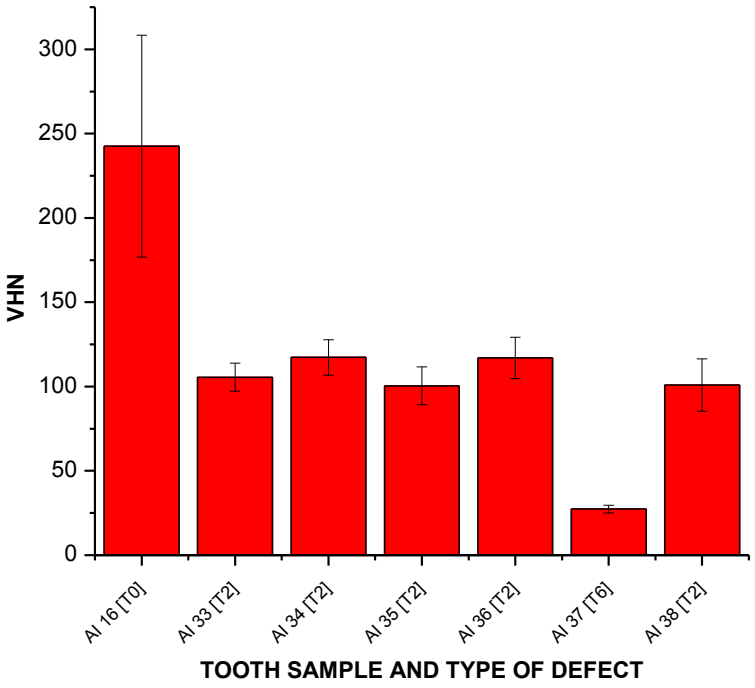

Figure 7-6 Mean enamel hardness for AI teeth with error bars representing the standard deviation for each tooth.

7.3.4 Statistical Analysis of Enamel Hardness

In this study, the mean VHN value for control teeth was based on a modified mean of 313.84 (95% CI; 296.78 – 330.90) as in Table 7-4. Table 7-5 compared the mean enamel hardness between different types of defect. Hardness of the enamel affected by MIH and AI were significantly lower than the enamel hardness of control teeth *(P* = 0.004). It was also noted that the hardness between the types of enamel defect was significantly different (*P* = 0.000) as shown in Table 7-6.

Group	Mean	df	95% Confidence Interval	
			Lower Bound	Upper Bound
Control	313.84	8.272	296.78	330.90
MIH	61.67	6.79	37.91	85.41
AI	126.53	9.14	102.26	150.81

Table 7-4 Mean hardness (VHN values) for Control, MIH and AI group based on modified mean.

Defect	Mean	df	95% Confidence Interval	
			Lower Bound	Upper Bound
Normal	278.21	10.24	255.22	301.20
White/cream	86.11	7.87	62.16	85.41
Yellow/brown	81.64	9.43	62.28	100.99
Hypoplasia – missing enamel	31.03	19.63	-2.91	64.98
PEB	41.60	11.66	16.49	66.71

Table 7-5 Mean hardness (VHN values) between the types of enamel defect based on modified mean.

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	8.023	449.602	.000
Group	2	9.811	9.9918	.004
Defect	4	59.947	29.104	.000

Table 7-6 Type III Test of Fixed Effects

Figure 7-7 indicates the interaction between the control, MIH and AI group to the different type of enamel defects. The control group considered as no enamel defect (normal) showed the highest enamel hardness amongst the group. Both the MIH and AI lines were not continuous, as there was an absence of hypoplasia-missing enamel and white/cream type of enamel defect in the group respectively.

From the graph, it clearly demonstrates decreased enamel hardness from the white/cream to PEB in the MIH group. The same finding established in AI group where the AI teeth affected with hypoplasia – missing enamel indicate a tremendous reduction in enamel hardness.

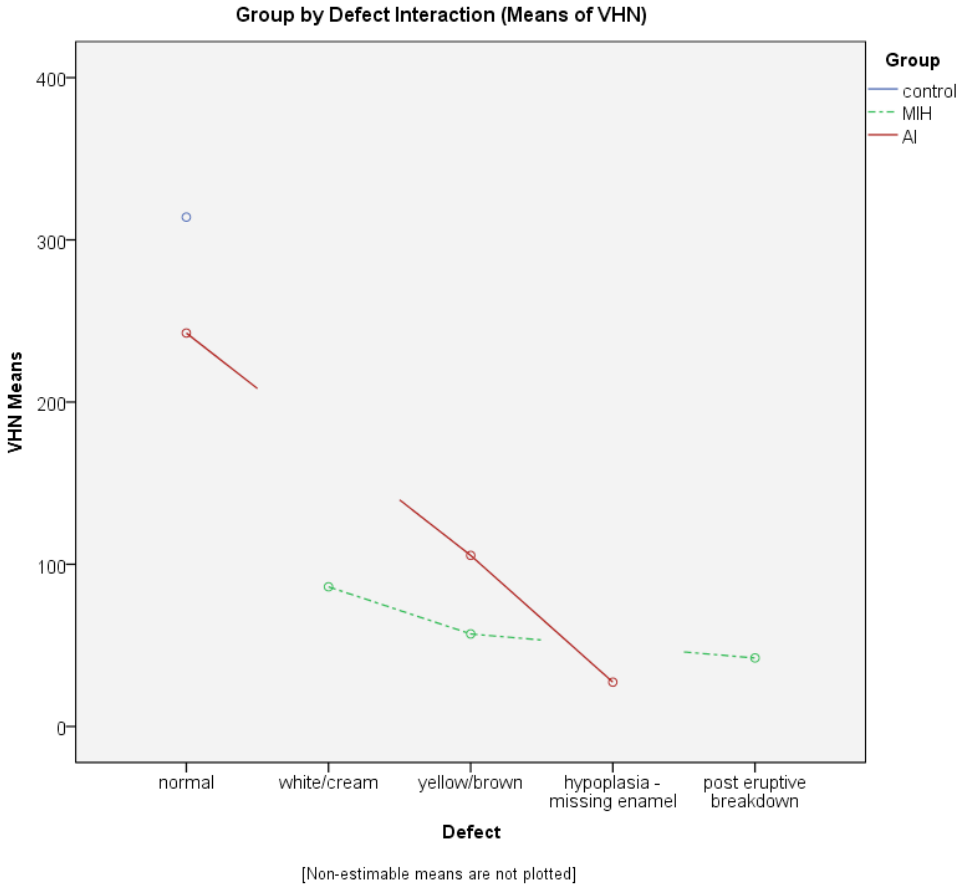

Figure 7-7 Interaction between each group and different type of enamel defect. Y- axis represents the mean VHN value and the X – axis exhibits the type of enamel defect.

7.4 Discussion

The results showed that the enamel affected in AI and MIH presented lower hardness values compared to the control and unaffected enamel. The enamel hardness for the control teeth were between VHN 222 to 360 which correspond to the previous study for enamel hardness (Gutierrez-Salazar and Reyes-Gasga, 2003, Gutierrez-Salazar and Reyes-Gasga, 2001).

The hypomineralised enamel showed lower hardness value. This finding is in line with a previous study carried out by Mahoney et al. (Mahoney et al., 2004b) and might be explained by the lower mineral content of enamel affected with AI and MIH. The hardness also differed between different types of defect, where the white/cream lesions showed the highest enamel hardness compared to the other type of lesions, as it is considered as the mildest type of MIH severity (Da Costa-Silva et al., 2011).

The enamel of normal teeth is a highly mineralised tissue containing hydroxyapatite. The enamel affected by AI has lost some of the normal architecture and the enamel rods are incompletely formed (Faria-e-Silva et al., 2011). The enamel hardness became very low for the teeth affected with PEB and when almost all the entire enamel layer is missing, which was notable in one of the AI samples. The enamel with yellow brown discolouration also appeared to be softer than the white cream enamel defects. This finding is comparable with a previous study when they concluded that mild MIH was associated with yellow opacity and brown enamel opacities were at higher risk for increase in severity of MIH (Da Costa-Silva et al., 2011).

However, the enamel hardness for the primary AI presented similar hardness values to the control and unaffected enamel surfaces. The teeth affected with hypocalcified type of AI showed a significant reduction in enamel hardness compared to the control teeth due to its reduction in mineral content. There is also possibility that the affected enamel has a similar hardness to the dentine as shown in previous study (Faria-e-Silva et al., 2011).

The hypoplastic type of AI also presented with reduction in the hardness values. Theoretically the hypoplastic type of AI should have higher hardness value compared to the hypocalcified AI as the condition is only affecting the amount of enamel matrix secreted and calcification occurs as usual. In this study, the enamel hardness was affected in both conditions. It could be due to presence of both enamel defect;

hypoplastic and hypocalcified, or due to the attrition of the enamel as the enamel layer is very thin which exposed to enamel breakdown.

The dramatic reduction in hardness of AI affected teeth, shown in this study may help to explain why hypoplastic teeth are traditionally difficult to restore, with clinicians reporting loss of both restorative material and tooth tissue. On the other hand, the underlying dentine may be more mineralized and potentially presenting with occluded tubules that can lead to lower bond strength (Faria-e-Silva et al., 2011).

It is clear that there is no relation between the DDE type of defect and hardness for AI teeth in this study, however, it must be remembered that the tested samples were collected from three patients with hypocalcified and hypoplastic AI. A patient may contribute more than one tooth sample. Therefore, the result could show similarities between the teeth from the same patient and these results need to be viewed with caution, and further testing with AI teeth from several subjects is required before any firm conclusions can be drawn.

The findings demonstrated that the hardness values in MIH were lower compared to the control teeth. This presents a challenging situation for the clinician in choosing the most appropriate restorative material and technique with which to manage this problem condition.

CHAPTER 8
RAMAN SPECTROSCOPY
(CHEMICAL VARIATION)

8 RAMAN SPECTROSCOPY

8.1 Background

Raman spectroscopy is a spectroscopic technique based on the inelastic scattering of monochromic light, usually from a laser source. Inelastic scattering means that the frequency of photons in monochromic light changes upon interaction with a specimen. Photons of the laser light are absorbed by the specimen and then reemitted. The frequency of the reemitted photons is shifted up or down in comparison with the original monochromic frequency, this is called the *Raman Effect*. This shift provides information about vibrational, rotational and other low frequency transitions in molecules. The Raman effect is based on molecular deformation determined by molecular polarisability (Banwell, 1983).

The Raman spectroscopy measures the characteristic Raman emission induced from molecules under monochromic laser irradiation, which excites the molecules and transforms them into oscillating dipoles. Those dipoles emit light of three different frequencies such as (Banwell, 1983) shown in Figure 8-1:

1. A molecule with no Raman-active modes absorbs a photon and the excited molecules return back to the same vibrational state and emit light with the same frequency as excitation source, this interaction is called an elastic ***Rayleigh scattering.***
2. A Raman-active molecule absorbs a photon, which at the time of interaction is in the basic vibrational state. Part of the photon's energy transferred to the Raman-active mode and resulting frequency of scattered light is reduced. This frequency is called ***Stokes frequency***.
3. A Raman-active molecule absorbs a photon, which at the time of interaction is in the excited vibrational state. Excessive energy of excited Raman active mode is released, molecules return to the basic vibrational state and the resulting frequency of scattered light goes up. This frequency is called ***Anti-Stokes frequency***.

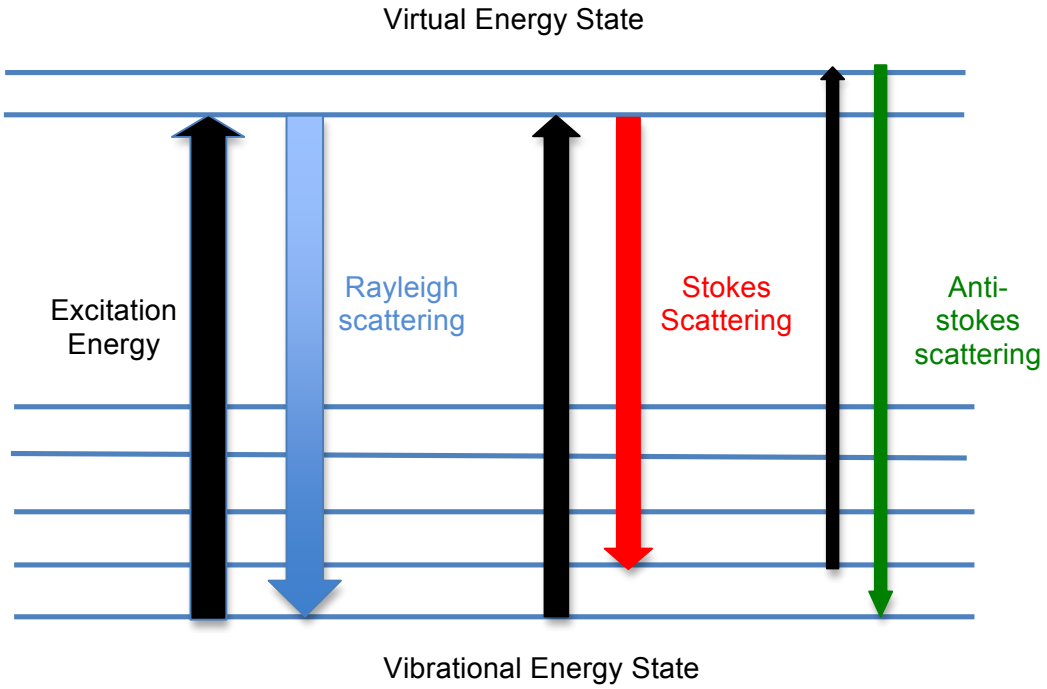

Figure 8-1 Energy level diagram showing the states involve in Raman signal.

In order to obtain the Raman spectrum, a laser is focused on the sample. The correct selection of the laser wavelength can be an important consideration for Raman spectroscopy. With modern equipment, often several laser wavelengths may be employed so as to achieve the best detection of the Raman signal.

For instance, many samples, especially those of an 'organic' or 'biological' nature will be quite fluorescent species. Exciting these samples with a laser in the green (532 nm) may promote this fluorescence, and may swamp any underlying Raman spectrum to such an extent that it is no longer detectable.

In this instance, the use of a laser in the red (633 nm) or near-infrared (NIR - 785 nm) may provide a solution. With the lower photon energy, a red or NIR laser may not promote the electronic transition (and hence the fluorescence) and so the Raman scatter may be far easier to detect.

Raman spectroscopy is a non-invasive method for obtaining information regarding molecular composition of material and for determination of structural information by molecular vibration analysis (Kinoshita et al., 2008). The Raman band positions or peaks are fingerprint to specific chemical groups. Therefore, they can be used for chemical identification and quantification. A reduction in the concentration of a

chemical group can cause a decrease in its spectral peak. Furthermore, the spectrum for the specimen can be recorded with limited sample preparation.

Raman spectra can be collected from a very small volume (< 1 μm in diameter). Water does not generally interfere with Raman spectral analysis. Raman signals are emitted in the form of light scattering and can be observed from all directions. Other advantages include non-destructive sample preparation, linear response to chemical concentration and easier spectrum analysis (Tsuda and Arends, 1997).

Raman studies have been used previously in dentistry to study enamel and carbonated apatite (Casciani et al., 1979, Nishino et al., 1981), dental adhesive resin (Ozaki et al., 1991, Suzuki et al., 1991), and recently, Raman technique has also been used for detection tooth caries when they found that there is low spectrum intensity of phosphate in the carious region (Ko et al., 2006, Kinoshita et al., 2008).

8.1.1 Carbonate Content in Enamel

Carbonate ion is considered as minor constituent in enamel. It can be incorporate into the hydroxyapatite while the apatite crystallites are forming. Concentration of carbonate in enamel is relatively high in the interior and decreasing towards the surface.

The distribution pattern of carbonate might reflect the events that occurred during the enamel formation. The carbonate in enamel originate from carbon dioxide produced by the ameloblasts cell as they advanced to the outer limit of the tissue during the amelogenesis, the amount of carbonate deposited in the mineral depend on the metabolic activity of the enamel-producing cells. The more activity they were, the more carbonate would be incorporated into the mineral. As their activity decrease toward the enamel surface, less carbon dioxide would be produced (Weatherell, 1975). Carbonate concentration level is highest in the forming enamel and begin to decrease at the early of maturation stage (a stage where the mineral concentration of developing enamel increase drastically) (Sydney-Zax et al., 1991).

The carbonate ion can lead to disorder of the apatite crystallites. Carbonate is smaller than phosphate and when it replaces phosphate will tend to reduce the crystallites dimension. It also can increase the ease with which the enamel mineral dissolves in acid (Weatherell et al., 1968).

Carbonate determination in enamel and dentine is important to study the dynamic of dental caries or developmental defect of these tissues. Few studies have shown the increase in carbonate content in hypomineralised enamel (Crombie et al., 2013, Fagrell et al., 2010, Crombie et al., 2010).

Therefore, the focus in this study is to investigate the carbonate content in MIH and AI teeth by means of Raman Spectroscopy.

8.2 Methodology

The Raman microspectroscopy (HR 800, Jobin Yvon, Horiba, Japan) was calibrated with known lines of silicone specimen with a characteristic band at 52.07 cm^{-1}. Spectra were obtained from all specimens using 20-mW red laser (HeNe) with a wavelength 632.8 nm, combine with slit width of 150 µm, a confocal hole of 400 µm and a 1800 grating and 50x magnification.

The specimen mounted horizontally in a glass slide using reusable putty-like pressure sensitive adhesive. Seven spectra at seven different points were taken surround the enamel. In MIH and AI group, seven spectra were carried out on the affected site of the enamel.

The spectra were all baselines corrected, smoothened and normalised at the phosphate peaks at 960 cm^{-1}. The spectra were overlaid for direct visual comparison. The peak maxima absorbances for phoshate (960 cm^{-1}) and carbonate (1070 cm^{-1}) peaks were recorded from the spectra.

Based on the Raman spectras, the ratio of the relative intensity between carbonate at 1070 cm^{-1} to phosphate at 960 cm^{-1} peak were then obtained to analyse the difference in mineral composition between the teeth in control, MIH and AI group.

8.2.1 Statistical Analysis

The data were then exported to Origin software. The statistical analysis for the carbonate to phosphate ratio for enamel in control, MIH and AI group was carried out using SPSS Version 22. The ratio data were analysed by Multilevel Model Analysis.

8.3 Results

The spectra for the Raman spectroscopy cover the shift range of 500 to 1500 cm^{-1}. All spectra were normalised based on the 960 cm^{-1}phosphate peaks, which was the most intense peak. The spectrum shows notable changes in peak intensities.

The greatest peak intensities were associated with the v_1, v_3 and v_4 phosphate at 960 cm^{-1}, 1043 cm^{-1} and 593 cm^{-1} respectively. The B-type carbonate peak noted at 1070 cm^{-1}, which represent the carbonate stretching mode. The phosphate v_1 peak, was a characteristic of the symmetric stretching mode of the tetrahedral phosphate.

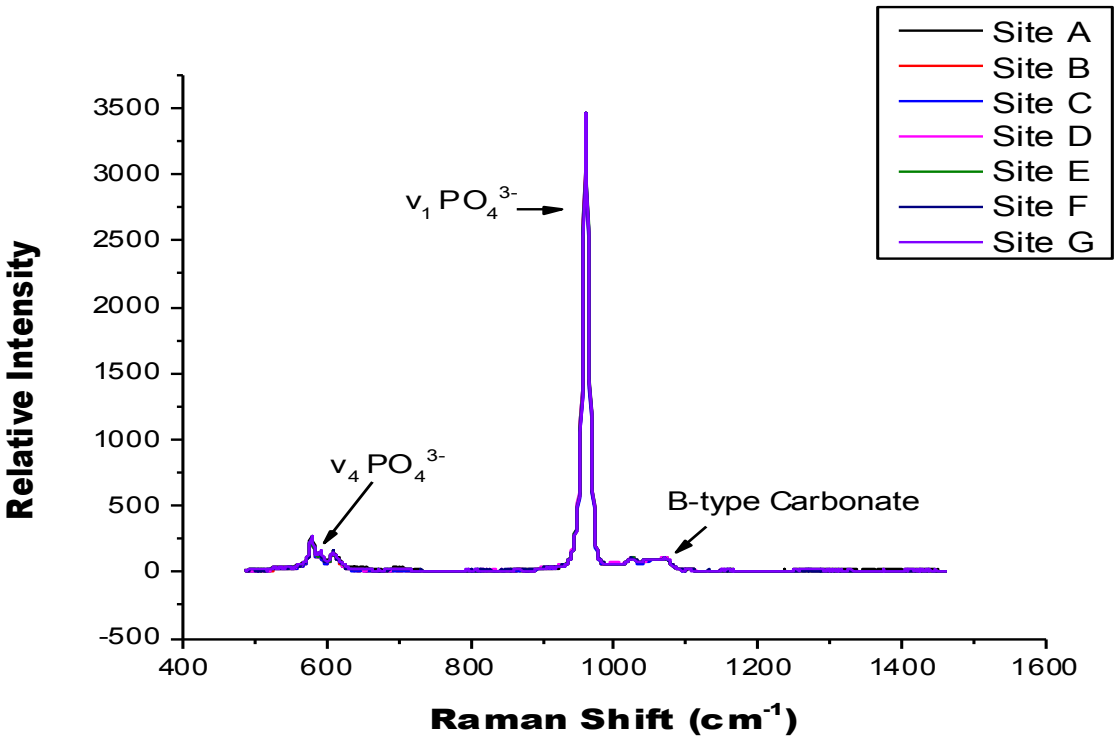

Figure 8-2 Micro Raman spectra for control tooth at 7 different sites

The spectra for the control tooth were taken at seven different sites. After the superimposition, the spectra appear homogenous and very regular (Figure 8-2). However, there were changes in the peak intensity in the MIH group taken on the affected surface of enamel where the most intense peak are still at 960 cm^{-1} but the carbonate peak at 1070 cm^{-1} show an increase in intensity.

The same changes in intensity were noted at the AI spectra for the permanent teeth but not in primary AI tooth. The spectra for the permanent AI showed additional peak which similar to the peak for the dentine. It show two characteristic parts, a region from 400 to 1100 cm^{-1} with an intense broad band at 980 cm^{-1} represent the mineral phase of dentine (carbonated hydroxyapatite) and region from 1200 to 3000 cm^{-1} for organic grouping vibration modes (amide and CH) which represent the collagen phase.

The mean carbonate to phosphate ratio for control teeth was 0.035 (± 0.006) as shown in Table 8-1.

Sample	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site7	Mean	SD
C93	0.039	0.035	0.051	0.052	0.042	0.046	0.047	0.045	0.006
C94	0.033	0.031	0.033	0.037	0.038	0.036	0.035	0.035	0.003
C95	0.028	0.025	0.028	0.029	0.027	0.027	0.030	0.028	0.001
C96	0.027	0.027	0.028	0.028	0.028	0.031	0.035	0.029	0.003
C97	0.030	0.030	0.029	0.033	0.030	0.030	0.031	0.030	0.001
C98	0.030	0.039	0.034	0.034	0.029	0.027	0.029	0.032	0.004
C102	0.036	0.034	0.038	0.035	0.042	0.039	0.037	0.037	0.003
C106	0.037	0.037	0.036	0.038	0.034	0.034	0.036	0.036	0.001
C107	0.033	0.036	0.038	0.032	0.035	0.034	0.034	0.035	0.002
C108	0.043	0.043	0.045	0.037	0.036	0.042	0.034	0.040	0.004
C109	0.035	0.034	0.031	0.030	0.033	0.028	0.029	0.031	0.003
C112	0.038	0.045	0.054	0.043	0.046	0.048	0.048	0.046	0.005

Table 8-1 Carbonate to phosphate ratio at seven different spectra for control teeth. Mean carbonate to phosphate ratio 0.035 (± 0.006)

MIH samples showed variation in the mean carbonate to phosphate ratio depending on the type of defect. The T1 lesion (white/cream) indicated the lowest and the highest mean of 0.042 and 0.090 respectively. However, the T2 lesion (yellow/brown) demonstrates between 0.093 to 0.172. The T8 type of defect (PEB) had a ratio between 0.105 and 0.117 (Table 8-2).

The carbonate to phosphate ratio for AI teeth was between 0.042 to 0.071 (Table 8-3).

Sample	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Mean	SD	Type of defect
MIH32	0.108	0.110	0.114	0.092	0.010	0.099	0.098	0.090	0.036	T1
MIH33	0.083	0.078	0.117	0.060	0.087	0.121	0.108	0.093	0.023	T2
MIH34	0.202	0.134	0.202	0.124	0.203	0.202	0.135	0.172	0.038	T2
MIH35	0.045	0.048	0.067	0.043	0.047	0.046	0.047	0.049	0.008	T1
MIH36	0.046	0.068	0.168	0.133	0.068	0.065	0.069	0.088	0.045	T1
MIH37	0.101	0.104	0.082	0.089	0.083	0.080	0.080	0.089	0.010	T1
MIH38	0.098	0.116	0.100	0.111	0.118	0.117	0.116	0.111	0.008	T8
MIH39	0.111	0.102	0.122	0.124	0.128	0.118	0.120	0.118	0.009	T8
MIH49	0.118	0.147	0.151	0.132	0.143	0.149	0.150	0.141	0.012	T2
MIH50	0.095	0.108	0.110	0.108	0.108	0.111	0.105	0.106	0.005	T8
MIH51	0.043	0.042	0.042	0.041	0.043	0.043	0.043	0.042	0.001	T1
MIH52	0.057	0.065	0.047	0.048	0.049	0.052	0.053	0.053	0.006	T1

Table 8-2 Carbonate to phosphate ratio at seven spectra and the mean values for MIH teeth. The right side of the table also shows the type of lesion for each tooth

Sample	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Mean	SD	Type of Defect
AI16	0.045	0.043	0.049	0.045	0.045	0.049	0.045	0.046	0.002	T0
Hypocalcified AI										
AI33	0.042	0.049	0.047	0.046	0.057	0.039	0.041	0.046	0.006	T2
AI34	0.063	0.102	0.063	0.071	0.060	0.074	0.052	0.069	0.016	T2
AI35	0.041	0.044	0.044	0.037	0.049	0.043	0.037	0.042	0.005	T2
AI36	0.062	0.076	0.087	0.059	0.051	0.050	0.038	0.061	0.017	T2
Hypoplastic AI										
AI37	0.067	0.070	0.071	0.072	0.075	0.073	0.072	0.071	0.003	T6
AI38	0.041	0.037	0.039	0.042	0.043	0.046	0.045	0.042	0.003	T2

Table 8-3 Carbonate to phosphate ratio and the mean values for AI teeth at 7 different sites.

Figure 8-4 indicates the summary of the carbonate to phosphate ratio comparing the control, MIH and AI group.

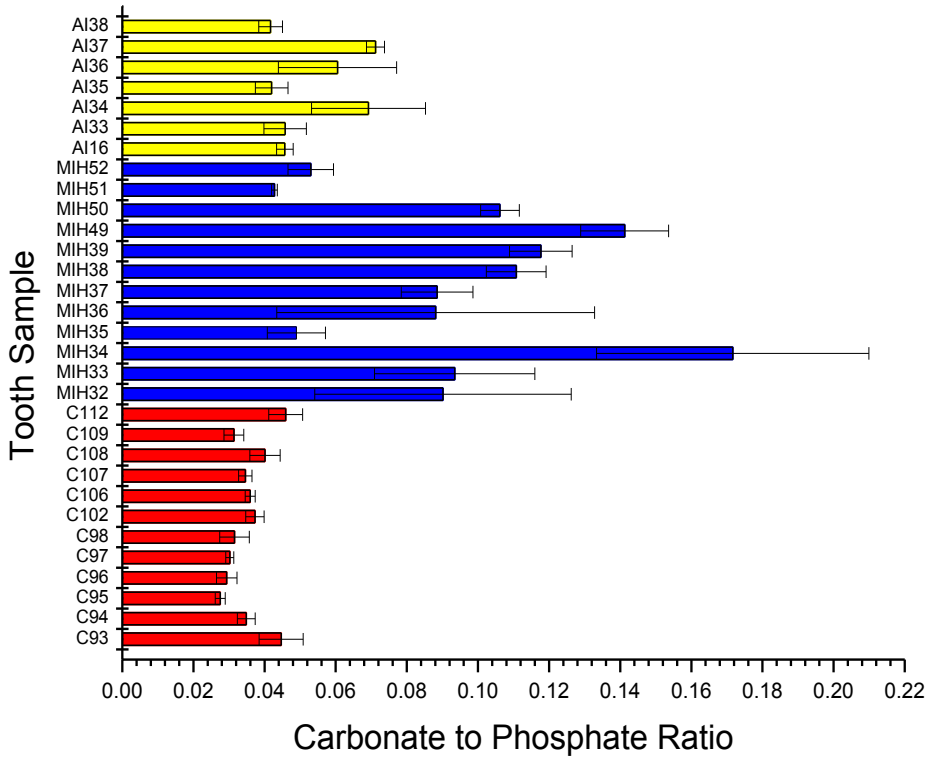

Figure 8-3 Summary of the mean ratio for carbonate (1070 cm^{-1}) to phosphate (960 cm^{-1}) in enamel for control, MIH and AI teeth. The red bars represent the control teeth, the blue bars represent the MIH teeth and the yellow bars indicate the AI teeth.

8.3.1 Statistical Analysis Carbonate to Phosphate Ratio

In this study, the mean carbonate to phosphate ratio for control teeth based on the modified mean was 0.037 (95% CI; 0.027 – 0.047), while the mean ratio in the MIH and AI groups were 0.102 (95% CI; 0.088 – 0.116) and 0.062 (95% CI; 0.048 to 0.076 as in Table 8-4. Table 8-5 indicates the mean carbonate to phosphate ratio between the different types of enamel defects

Group	Mean	df	95% Confidence Interval	
			Lower Bound	Upper Bound
Control	0.037	9.434	0.027	0.047
MIH	0.102	8.777	0.088	0.116
AI	0.062	10.888	0.048	0.076

Table 8-4 Mean carbonate to phosphate ratio for Control, MIH and AI group based on modified mean.

Defect	Mean	df	95% Confidence Interval	
			Lower Bound	Upper Bound
Normal	0.037	9.434	0.027	0.047
White/cream	0.066	9.236	0.052	0.080
Yellow/brown	0.094	10.981	0.084	0.104
Hypoplasia – missing enamel	0.076	20.787	0.060	0.092
PEB	0.100	11.135	0.085	0.114

Table 8-5 Mean carbonate to phosphate ratio between the types of enamel defect based on modified mean.

The carbonate to phosphate ratio was significantly high in MIH and AI teeth compared to the control group. The ratio also showed a significant difference between each type of enamel defect with P value of 0.000 as shown in Table 8-6.

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	9.826	385.547	.000
Group	2	198.697	185.405	.000
Defect	4	10.981	101.746	.000

Table 8-6 Type III Test of Fixed Effects

8.4 Discussion

Raman spectroscopy provides a biochemical characterisation of hydroxyapatite, the major mineral component of tooth enamel and allows a thorough molecular analysis of mineralised dental tissue. The information provided is in the form of curves representing the intensity of the signal according to the frequency and mathematical analysis. It was believed that the reduction in Raman polarisation is due to increase scattering and alteration in the degree of hydroxyapatite crystal orientation with demineralisation (Ko et al., 2006). In sound enamel, the intense peak at 960 cm^{-1} is associated with phosphate (PO_4^{3-}) stretching vibration in the mineral apatite component (Zavala-Alonso et al., 2012, Ko et al., 2006) and B-type carbonate peak at 1070 cm^{-1} (Zavala-Alonso et al., 2012).

In the enamel, carbonate can be very easily incorporated via ionic substitution replacing the hydroxide ion or phosphate ions which known as carbonatoapatite. The incorporation of the carbonate ion effect the apatite crystal leads to instability of the apatite (Robinson et al., 1995). The substitution of the carbonate ions replacing the hydroxide ion and phosphate ion known as A-type and B-type substitution which caused expansion or contraction of the apatite lattice respectively (Spizzirri et al., 2012). Approximately 11 wt.% of carbonate replaces hydroxide ion and the remaining replaces phosphate ion (Clasen and Ruyter, 1997).

Carbonate content is known to vary in concentration depending on the location in the enamel. The concentration of carbonate gradually increase from the enamel surface to the inner enamel near the DEJ (Xu et al., 2012b). The interest about the incorporation of carbonate into enamel commenced when the enamel affected by MIH found to have higher carbonate content than the normal enamel (Crombie et al., 2010).

Analysis of the carbonate content was considered as a difficult problem in chemistry and involved a complicated procedure. Conventionally, carbonate determination can be obtained by employment of an acidic digestion of the apatite crystal releasing the carbon dioxide gas, which then can be calculated by weight (Weatherell et al., 1968).

However, the used of Raman spectroscopy in the study of molecular structure provides detail information at molecular level and is a non-destructive and rapid procedure. There have been three reported methods to determine carbonate content using Raman spectroscopy. These methods are to monitor: i) changes to the phosphate line width,

which increase when carbonate concentration rises or also known as full-width-at-half-maximum (as a result of reduce crystallite size and higher disorder), ii) the intensity ratio of the carbonate band to the phosphate band, iii) the area ratio of the carbonate band relative to the phosphate band and each of the method resulted in a different determination of carbonate content (Spizzirri et al., 2012).

In this study, the method used was analysing the intensity ratio of the carbonate band (1070 cm^{-1}) to phosphate (960 cm^{-1}). The same method was used by Xu et al. to study the distribution pattern of carbonate in the enamel (Xu et al., 2012a). The high carbonate intensity in MIH was in agreement with the previous study by Crombie et. al., which showed that the enamel effected with MIH has higher carbonate content than normal enamel (Crombie et al., 2013). The presence of carbonate in enamel and also dentine increased their solubility and therefore affected their susceptibility to dental caries. This is perhaps the underlying reason behind the risk of developing caries in MIH and AI teeth.

Children with MIH are known to have hypersensitivity problem, especially in PEB lesion. They will tend to minimise any contact to the affected teeth including during tooth brushing which then leads to oral hygiene difficulty. The structure of enamel in affected teeth is usually intact until the eruption, but it is actually soft and porous and at high risk of gradual enamel breakdown. This will leave the enamel surface as a rough surface and plaque retentive. Teeth with MIH has been shown to be associated with caries formation (Jeremias et al., 2013).

Studies comparing the carbonate content in AI teeth are sparse compared to MIH. In this study, even though the carbonate content in AI group was significantly higher than normal enamel, the result is actually a combination between different types of AI, which are hypoplastic and hypocalcified. This result is different from the previous study. A study has shown that the carbonate content in hypocalcified type of AI was found to be similar with the normal enamel (Wright et al., 1993). Another study by Wright et al. shown that there is no significant different in the carbonate content between the hypoplastic (smooth surface) AI and the normal enamel (Wright et al., 1991). They used the conventional method to determine carbonate content in the enamel and the samples were from a patient with hypoplastic type of AI (smooth surface).

The recent study regarding elemental analysis in AI teeth, found an increase of carbon level in teeth affected with hypocalcified AI compared to healthy control teeth using energy dispersive x-ray spectroscopy (EDX) (El-Sayed et al., 2010).

The difference in the result may be associated with different technique used and type of the enamel defect. From this study, the highest carbonate to phosphate ratio was found in the hypoplastic AI with missing enamel.

In this study, both MIH and AI showed high standard deviation, as shown by the error bars in Figure 8-3. This is due to the difficulty in focussing the image tooth sample to obtain Raman spectra as most of the enamel defect had rough and uneven surface. The focussing became more difficult if the enamel defect involve the cusp with PEB. A sharp image is important to gain a consistent Raman spectra or it will lead to inconsistency and difficulty in analysing the outcome data.

CHAPTER 9
SCANNING ELECTRON MICROSCOPY
(ENAMEL ULTRASTRUCTURE)

9 SCANNING ELECTRON MICROSCOPY

9.1 Background

A scanning electron microscope (SEM) is a type of electron microscope that produces images of a sample by scanning it with a focused beam of electrons. It allows to image samples with larger depth of field and allows more of a specimen to be in focus at one time. This technique uses an electron beam to scan across the surface of the sample resulting in the electron beam interacting with atoms present on the surface of the sample. Previous study used light microscopy which has limited depth of focus and resolution and only useful for more shallow surface features (Boyde, 1975) .

SEM has been used extensively to study the ultrastructure of the enamel (Boyde, 1975, Jalevik et al., 2005, Azinovic et al., 2003). Sabel *et al.* used polarized light and SEM and polarized light microscope (POLMI) to investigate the morphology of hypoplastic enamel in primary teeth. The study concluded that the hypoplastic enamel had a rounded appearance. A bending of the prisms in the rounded borders was observed both in POLMI and SEM. These was due to the ameloblasts in the area of the enamel hypoplasia have not formed any more enamel. Eventually the unaffected ameloblasts having no neighbouring ameloblasts reacted by successively forming a rounded border (Sabel et al., 2010).

A study on mouse models to investigate shear bond strength in AI. In the study, it compares the etching characteristics and bond strength of enamel in wild type (WT) mouse with those having mutation in amelogenin (*AmelxKO*) which similar to X-linked AI in human and those lacking matrix metalloproteinase-20 (*Mmp20KO*) which mimics human MMP20 mutation AI. The study suggested eliminating the traditional use of acid etching in the patient with genetic variant as it may possibly decrease the failure rate of a restoration. They found the unetched enamel in AmelxKO was naturally rougher than the unetched WT and both the unetched AmelxKO and Mmp20KO appear rougher than the etched surfaces. The study also shown that the use of self-etching bonding system in AmelxKO and MMp20KO resulted in higher shear bonding strength compared to the etch-and rinse bonding system (Pugach et al., 2011).

Tobias *et al*, studied the hypomineralised enamel under SEM to investigate the morphological properties of the defective enamel. They found that the hypomineralised enamel appear dark in low magnification and had vague prism borders and crystals at higher magnification. There are also clear inter rod spaces. In contrast with the normal

enamel, which appears white and bright and well, organised distinct enamel rods and crystals. Therefore, the hypomineralised enamel appeared more porous than the normal enamel (Fagrell et al., 2010).

9.2 Methodology

Each stored tooth was mounted on a metal rectangular block using hot, Beeswax. Firmly mounted tooth was then sectioned using a table top cut-off grinding machine (Accutom-50, Struers UK) as shown in Figure 9-1. The tooth sample was sectioned through the enamel defect depending on the location of the enamel defect (Figure 9-2).

Figure 9-1 Cut-off grinding machine with the cut-off wheel used to cut the tooth sample

Prior to the SEM imaging, the tooth samples were demineralised by etching with 37% phosphoric acid for 30 seconds and rinsed with distilled water and dehydrate at room temperature for 24-hours. The samples were mounted onto aluminium pin stub using two tubes Araldite (Agar Scientific, UK) and sputter coated with gold/palladium (Polaron E5000, Quorum Technology, UK) for imaging. The samples were then examined in a FEI XL30 FEGSEM (FEI UK, UK), operating at 5 kV.

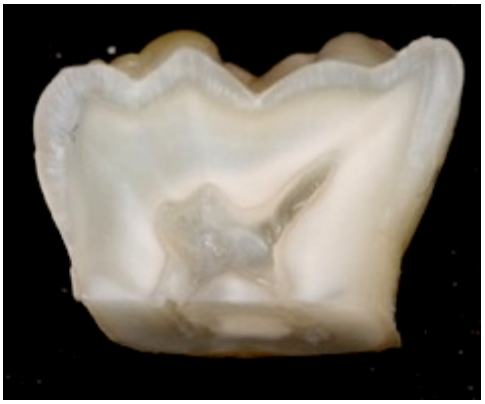

Figure 9-2 Longitudinal section of a tooth for SEM imaging

9.3 Result

9.3.1 SEM for control permanent teeth

For the control teeth, the SEM images are shown in Figure 9-3 – 9-7. The etched enamel of the control teeth shows a typical etching pattern either Type I or II pattern. Type I pattern was characterized by deep central excavation and prominent margin where the enamel rod core is removed leaving the peripheries (Figure 9-3). Type II pattern showed the peripheral regions of the enamel rod were dissolved, leaving the rod core intact (Silverstone et al., 1975). It is also known as 'keyhole appearance' (Figure 9-4).

In normal enamel, the orientation of the enamel prisms was without interruption and well-organised (Figure 9-5). A typical HSB can be seen at the middle third of the enamel layer (Figure 9-6).

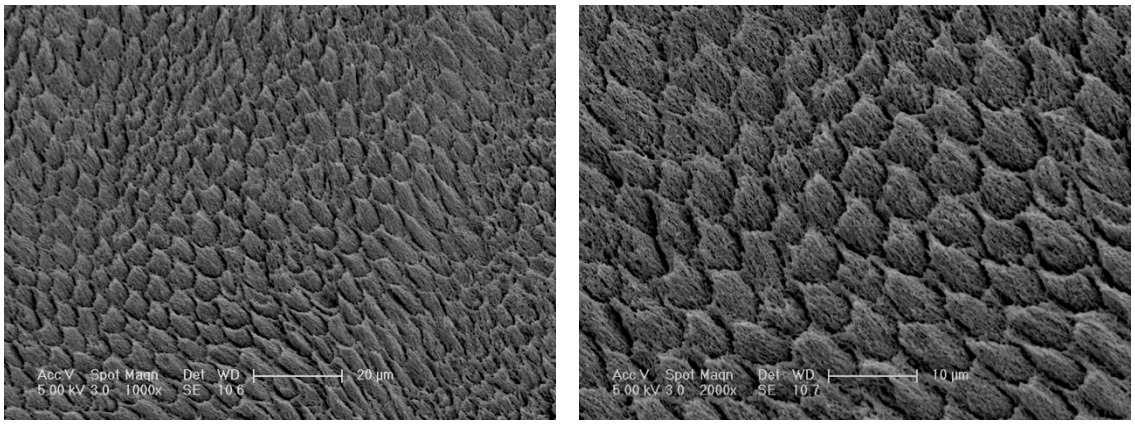

Figure 9-3 Type I pattern of enamel that has been acid etched characterised by deep central excavation, prominent margin and the prism cores were removed, leaving the prism peripheries intact.

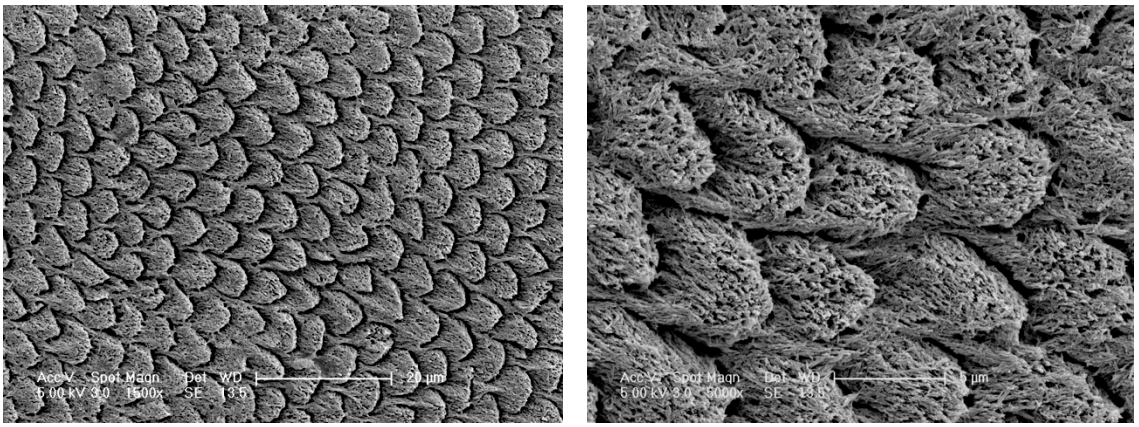

Figure 9-4 Type II pattern showing the protrusion of the enamel prism core (keyhole appearance) and enamel prism core with gap separating them corresponding to the inter prism space.

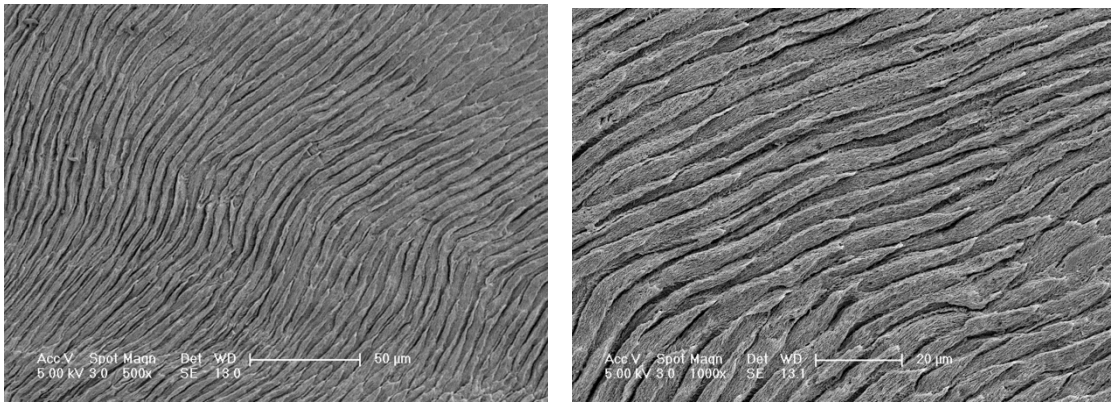

Figure 9-5 Homogenous orientation of enamel prism.

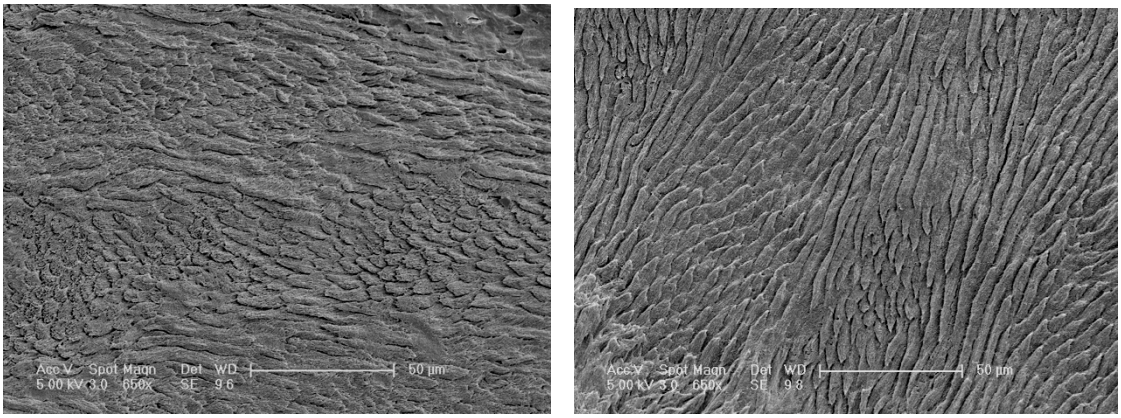

Figure 9-6 Hunter-Schreger bands

At higher magnification, the crystallites forming the enamel rod are arranged as a well-organised needle-like structure as shown in Figure 9-7.

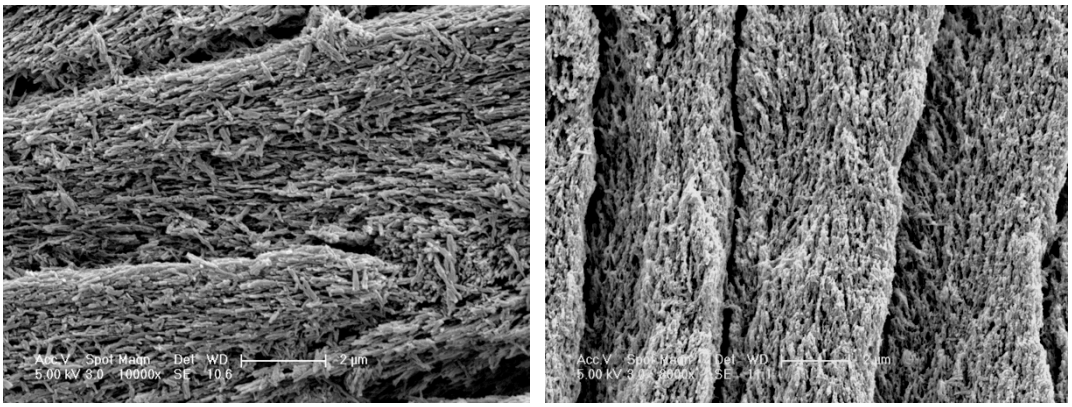

Figure 9-7 Well-organised thin needle-like crystallites forming the enamel rods with well demarcated boundaries separating each rods.

9.3.2 SEM for MIH teeth

9.3.2.1 White/cream Type of Defect

Six MIH teeth with white/cream type of defect were examined for SEM imaging. Figure 9-8 demonstrates the macroscopic features of the teeth with white/cream enamel defect after longitudinal section. The enamel defects are well-demarcated opacities and some of the teeth had been restored with different type of restorations such as fissure sealant, composite resin and amalgam. After the teeth were dissected, it can be clearly seen that all of the restorations were placed on the defective enamel.

Figure 9-8 Macroscopic appearance of the enamel in MIH teeth with white/cream type of defect after longitudinally sectioned. The red arrows indicate the location of the defect for SEM imaging. All of the teeth show well-demarcated opacities extending from the surface of the enamel to the DEJ. Dental restoration using composite resin, amalgam, glass ionomer and fissure sealant were noted at A, B, C, E and F respectively.

Microscopically, the enamel prisms appeared less organised, irregular, with increased inter rod spaces leading to porosity. There were areas where the enamel was covered by a structureless layer (Figure 9-9).

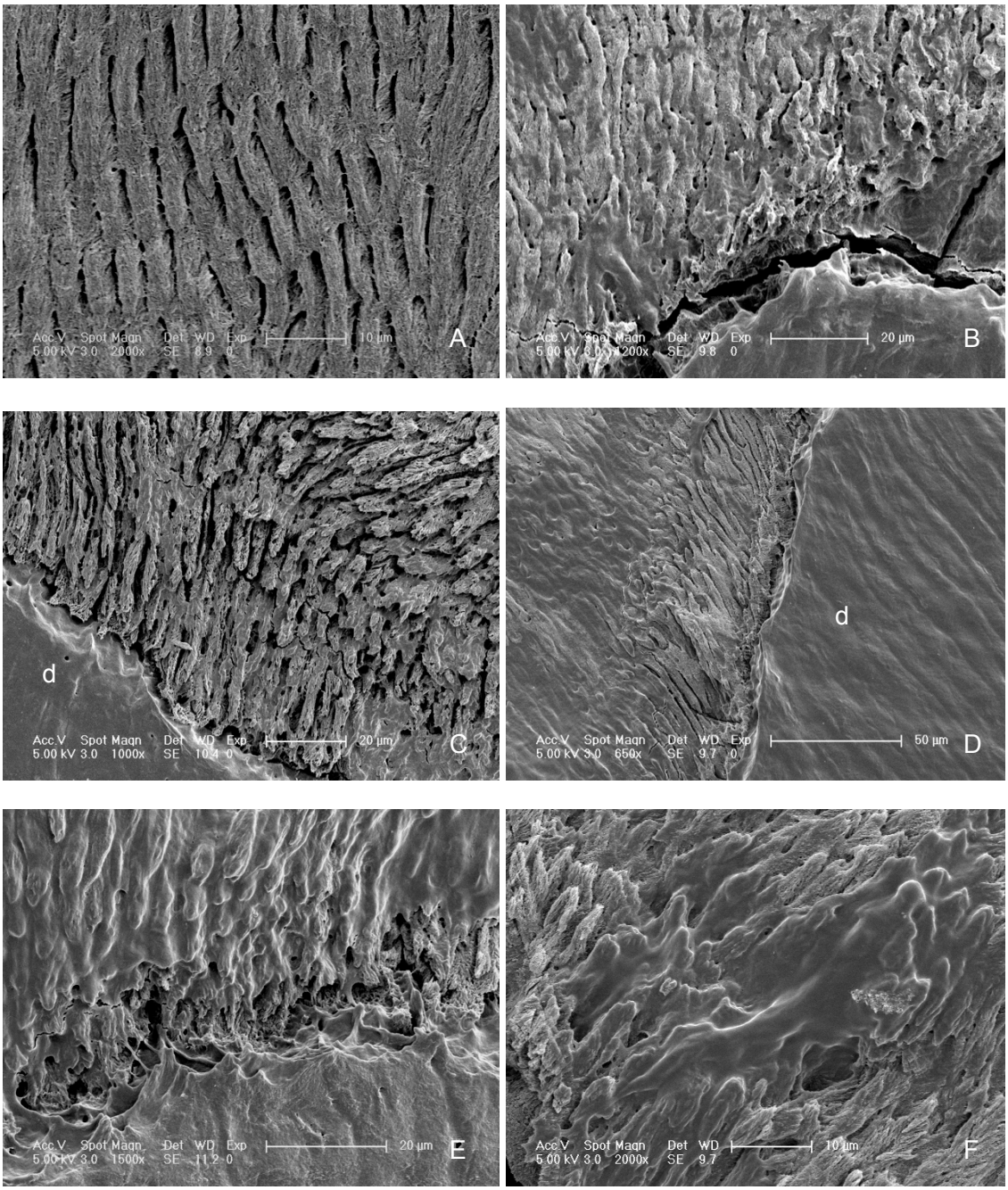

Figure 9-9 SEM images of etched enamel. Each image corresponds to the macroscopic picture for six MIH teeth with white/cream type of defect. A; Porous enamel rod with present of wide interred space. B; Disorganized enamel rod and loss of typical enamel rod structure after etched. C; Very porous enamel rod near the DEJ. D, E and F; Enamel rod obscured by featureless and amorphous layer at different magnification. d, dentine.

9.3.2.2 Yellow/brown Type of Defect

Four MIH teeth affected with yellow/brown defect viewed under SEM. Figure 9-10 shows the images of one of the teeth, as the presentation was similar.

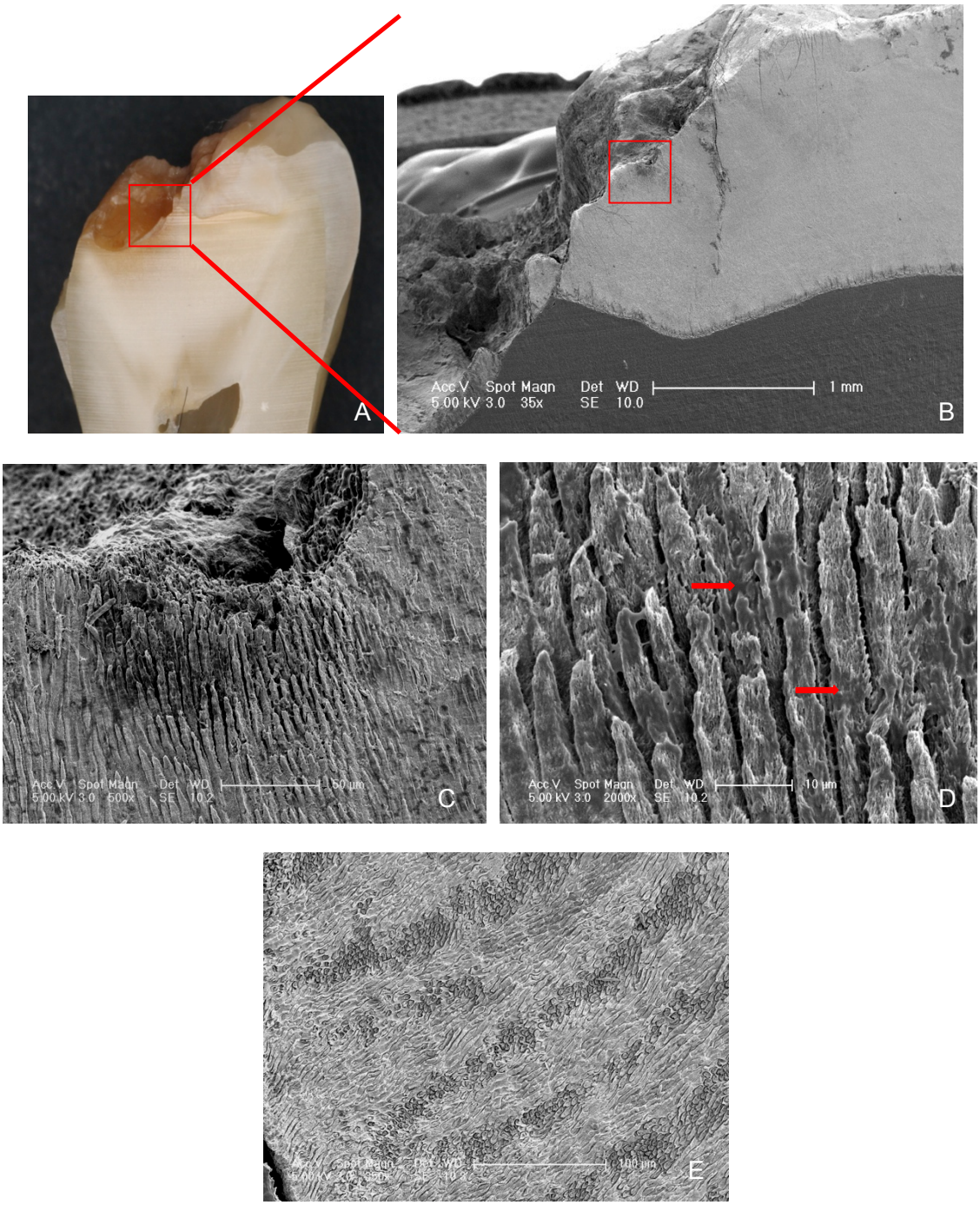

Figure 9-10 SEM images for MIH tooth (MIH 33) affected with yellow/brown type of enamel defect. A; Macroscopic feature of a longitudinally dissected tooth. The squares mark the area where the scans were performed as shown in B. C, D; Image taken indicated by the square mark in image B, confirming disintegration of the enamel in the disturbed area near the surface with pronounced porosity and presence of structureless layer covering the enamel rod and crystallites, marked by red arrow. E; Hunter Schreger's band found on unaffected enamel region.

9.3.2.3 PEB Type of Defect

Macroscopically, the teeth affected with PEB showed enamel breakdown especially at the cuspal region as shown in Figure 9-11. There was clear evidence of gap formation between the enamel and the restorative material and showed greater porosity and the presence of a structureless layer (Figure 9-12 and 9-13).

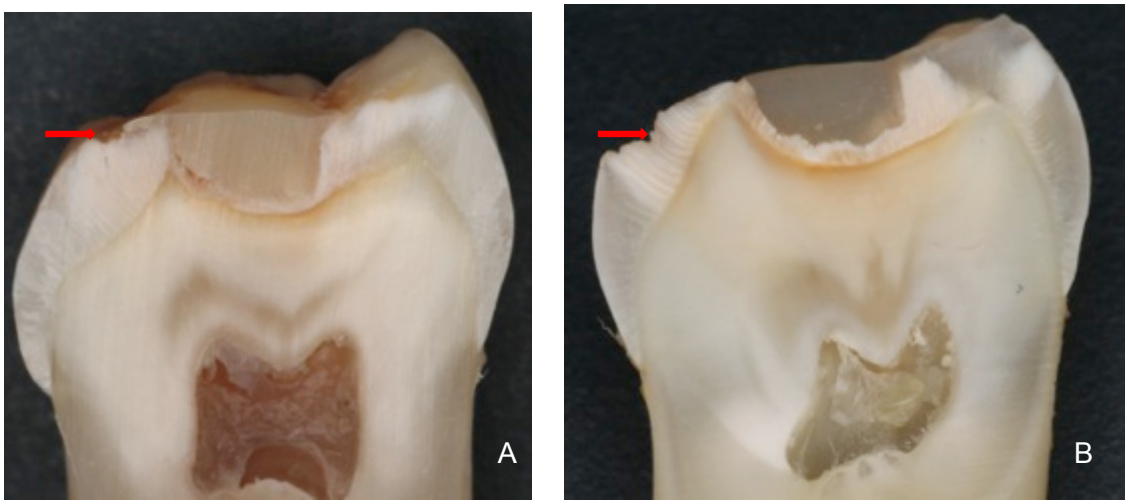

Figure 9-11 Macroscopic features of two MIH teeth (MIH 38 & MIH 39) after longitudinally dissected. Red arrows show the missing cusp due to the enamel breakdown. Both teeth were restored on the affected enamel with demarcated opacities extending from the enamel surface toward the DEJ.

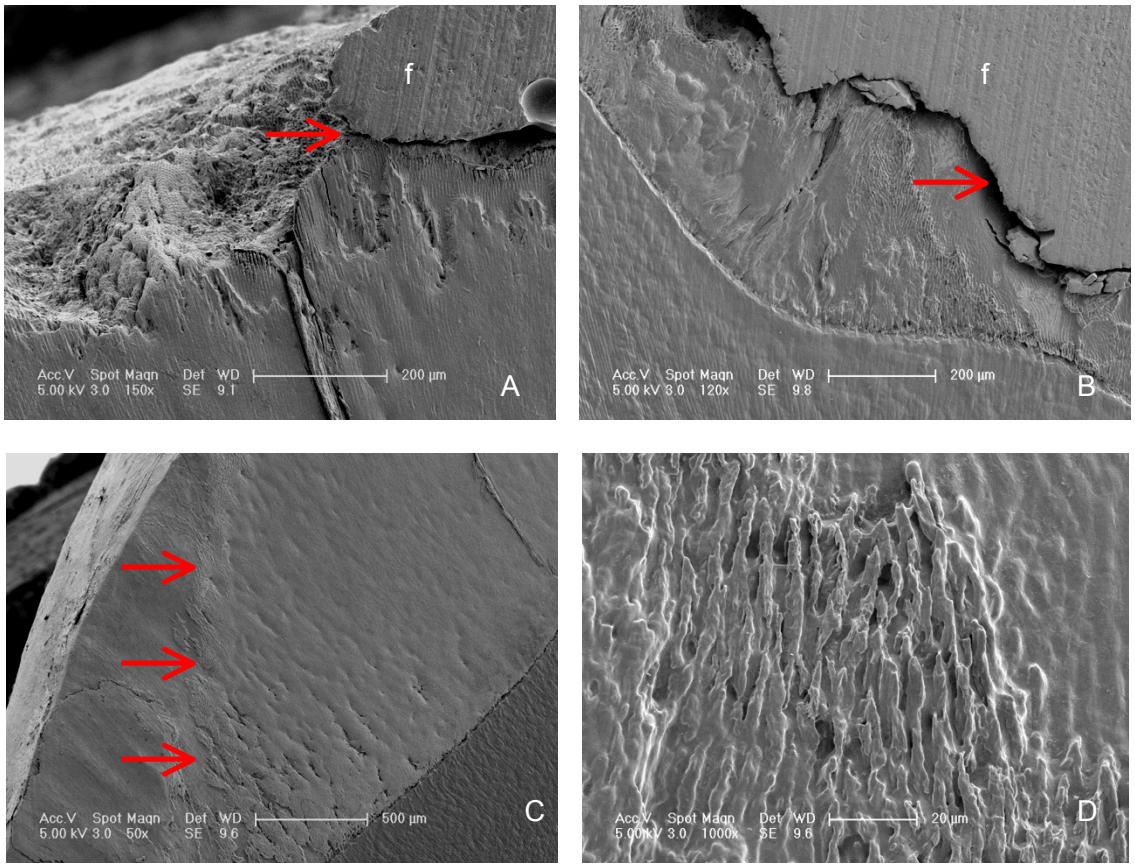

Figure 9-12 SEM images for teeth MIH 38 with PEB. A, B; Image of enamel surface show rough and uneven contour. The red arrows indicate the presence of huge gap between the restorative material, f and the hypomineralised enamel. C; Arrows indicate the border between the normal enamel and the hypomineralised area. D; Enamel rods covered by structureless layer that unaffected by the phosphoric acid during the demineralized process.

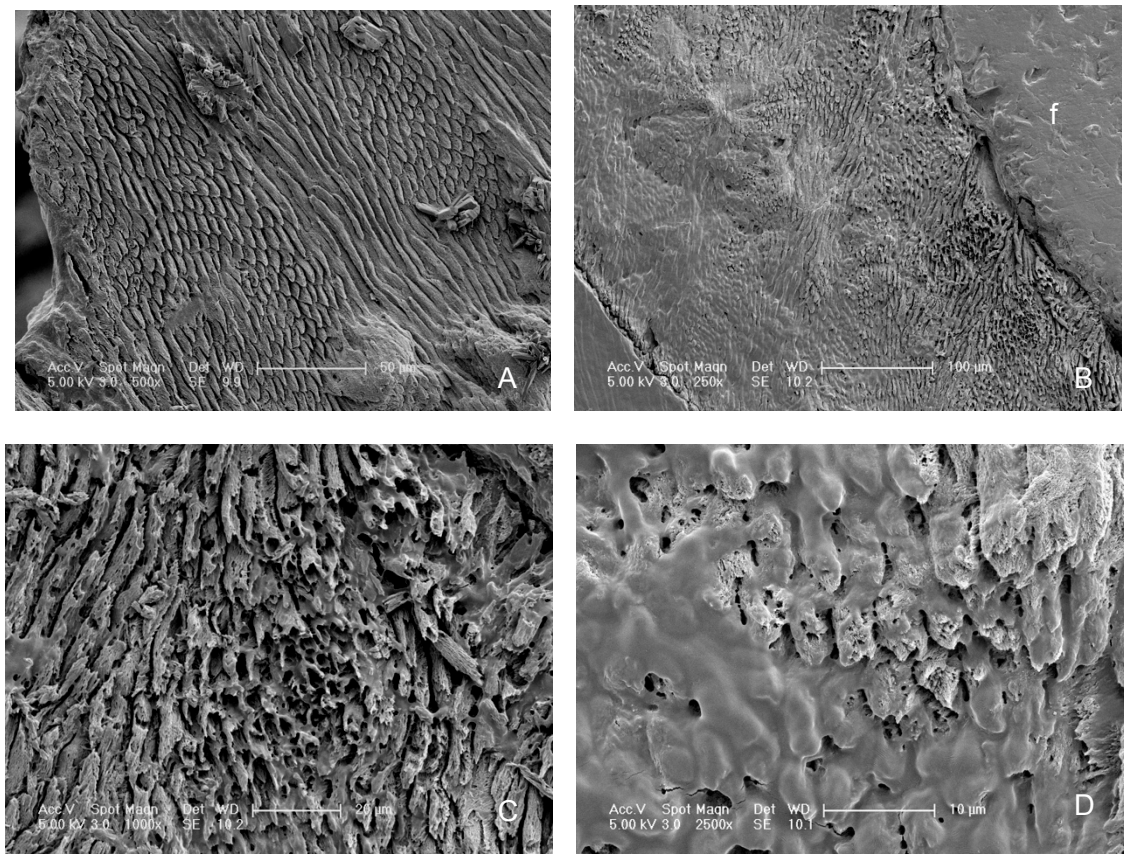

Figure 9-13 SEM images for MIH tooth (MIH 39) affected with PEB. A; Image shows the structure of the enamel near the surface indicates that the lesion had extend to the middle third of the enamel layer due to the presence of Hunter Schreger's band. B; Restorative material, f was placed on a defective enamel. C; Disorganised and porous enamel underneath the restoration. D; At higher magnification shows some of the enamel rod uncovered from the structureless layer.

9.3.3 SEM for AI teeth

The primary AI tooth, showed no difference compared to the normal enamel structure with well-organised enamel prisms (Figure 9-14). However, the microscopic features for the permanent enamel teeth with AI had a totally different characteristics when viewed under SEM.

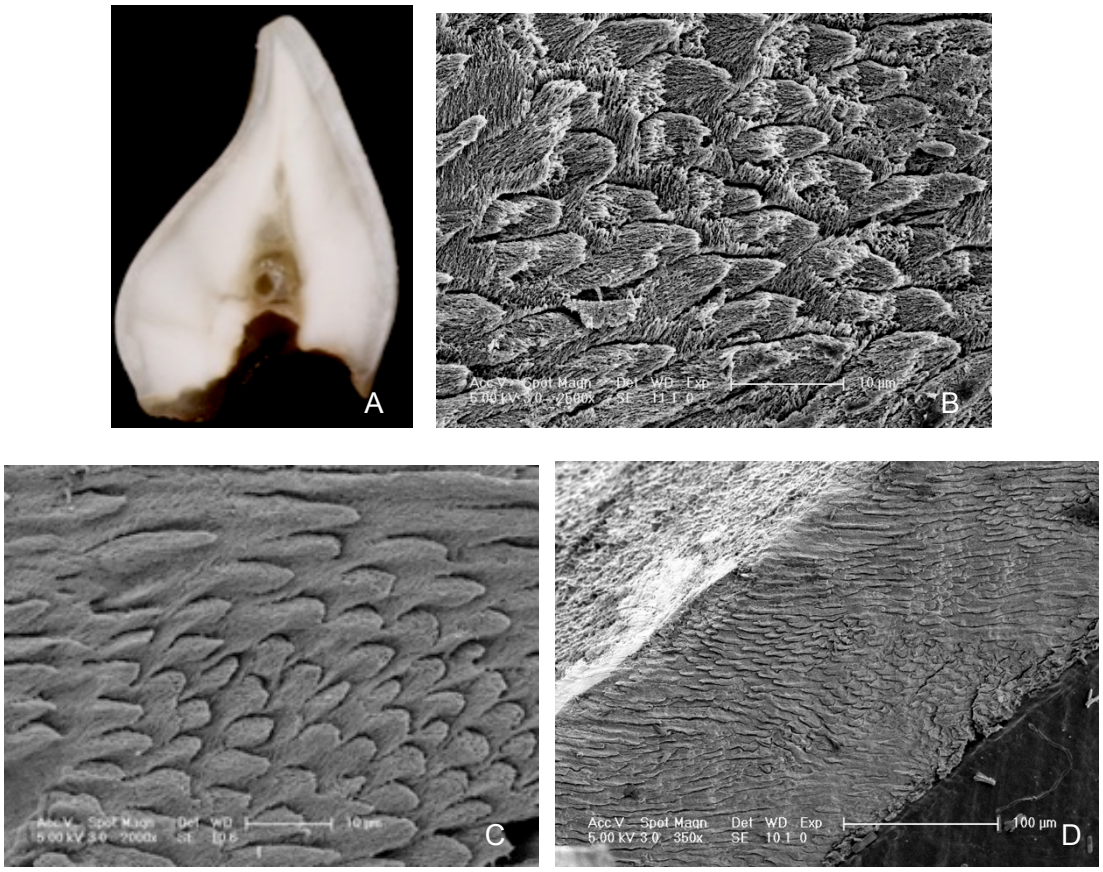

Figure 9-14 SEM images for primary AI teeth (AI 16). A; Macroscopic feature of the longitunally dissected tooth shows normal thickness of the enamel with no opacities. B, C, D; Well-organised enamel structure with typical appearance of enamel rod after demineralised with phosphoric acid.

The hypomineralised type of AI teeth show a distinct layer of enamel, the outer layer was more translucent and inner layer was more opaque when viewed clinically after being sectioned longitudinally (Figure 9-15). Under the SEM, there was a transition between the outer and inner layer where the outer layer demonstrated clearer, disorganised and porous enamel prism. Conversely, the inner layer, which appeared as dark zone at low magnification, was an amorphous structureless layer covering the enamel rods (Figure 9-16 and 9-17).

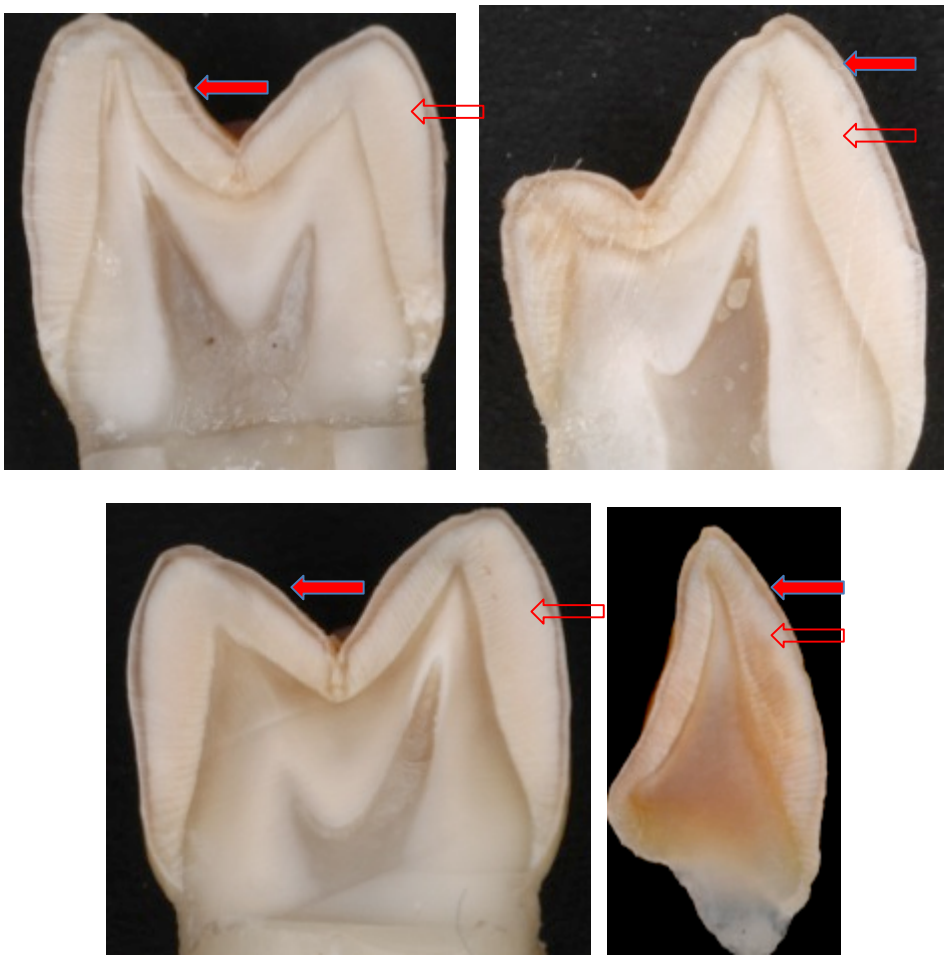

Figure 9-15 Macroscopic features of the AI teeth, which have been diagnosed as hypocalcified type of AI. These teeth are from the same patient. Note the two distinct layers in the enamel marked with the arrow. The outer layer (red arrow) appeared more translucent while the inner layer (open arrow) appeared more opaque.

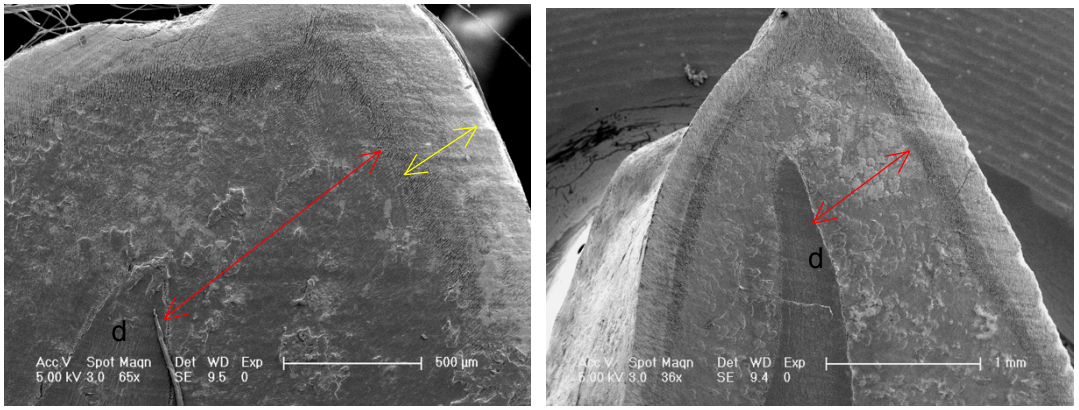

Figure 9-16 At low magnification, the enamel demonstrates a clear separation between the two layers of the enamel between the outer (yellow arrow) and inner layer (red arrow). The inner layer appeared darker than the outer layer. d, dentine.

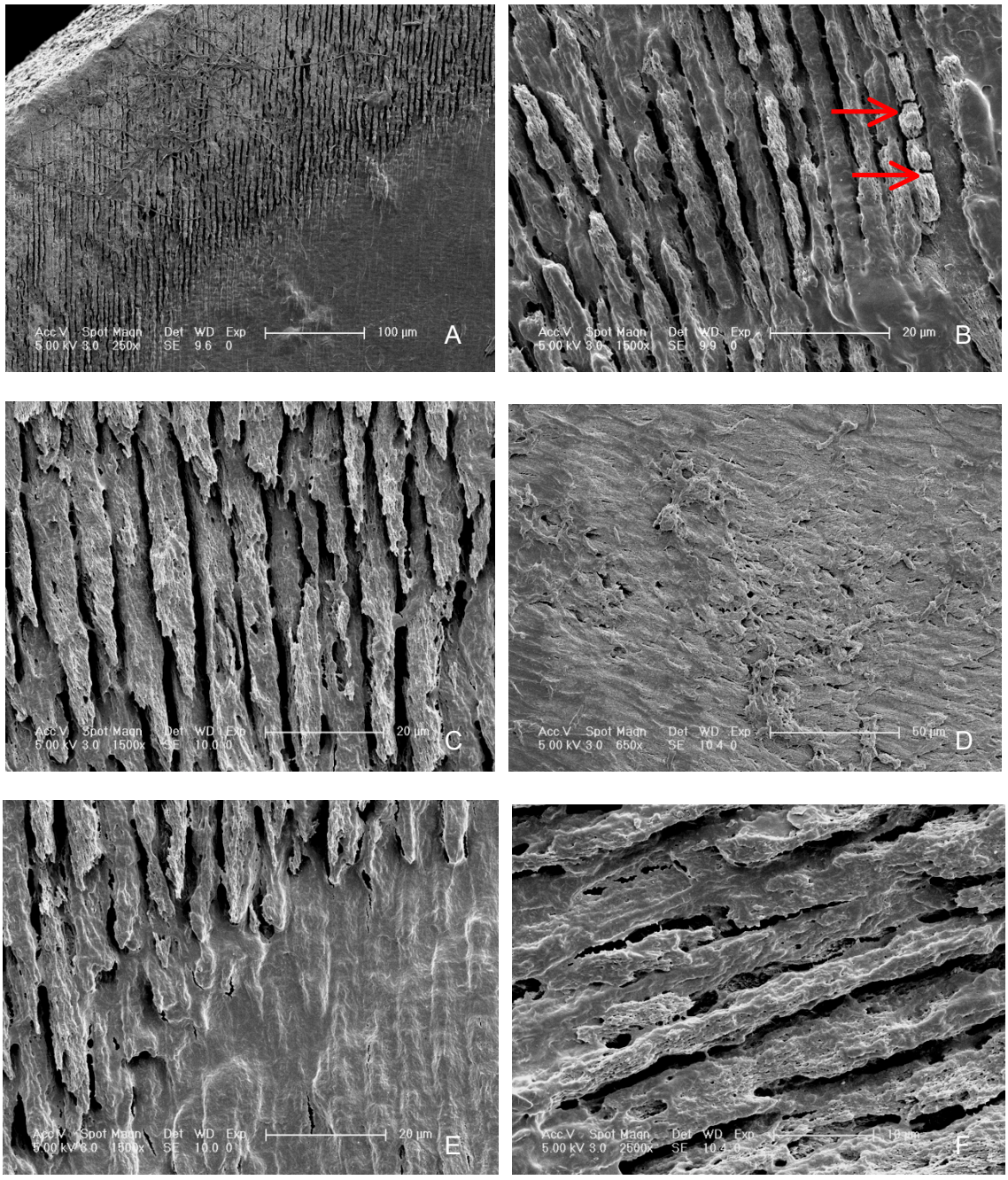

Figure 9-17 A; Image showing the distinct two layers of enamel in hypomineralised AI. B; Filamentous pattern of the enamel prism with interruption along their axis. C; The appearance of the enamel rod at the outer part of the enamel. D; Image of the inner part of the enamel shows lack of visible enamel rod and highly disordered. E, F; The enamel rods appeared as 'glass-like' appearance where the individual crystal seemed to have fused each other.

Macroscopically, the hypoplastic AI teeth showed uneven enamel thickness with rough enamel surface (Figure 9-18). At low magnification, the enamel appeared rough and defective and some parts left with thin layer of enamel (Figure 9-19). The microscopic features demonstrated disorganised enamel rods and crystallites, unclear enamel rod boundaries and presence of 'glass-like' appearance (Figure 9-20 and 9-21).

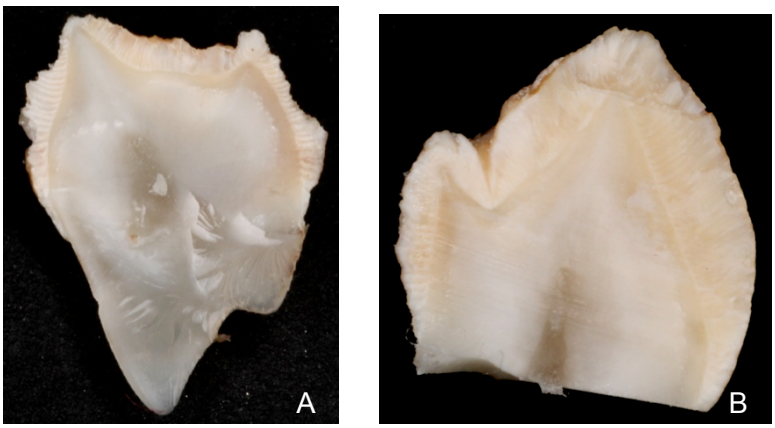

Figure 9-18 Macroscopic features of the AI teeth with hypolastic type of AI. The enamel appears very thin especially in A and rough surface.

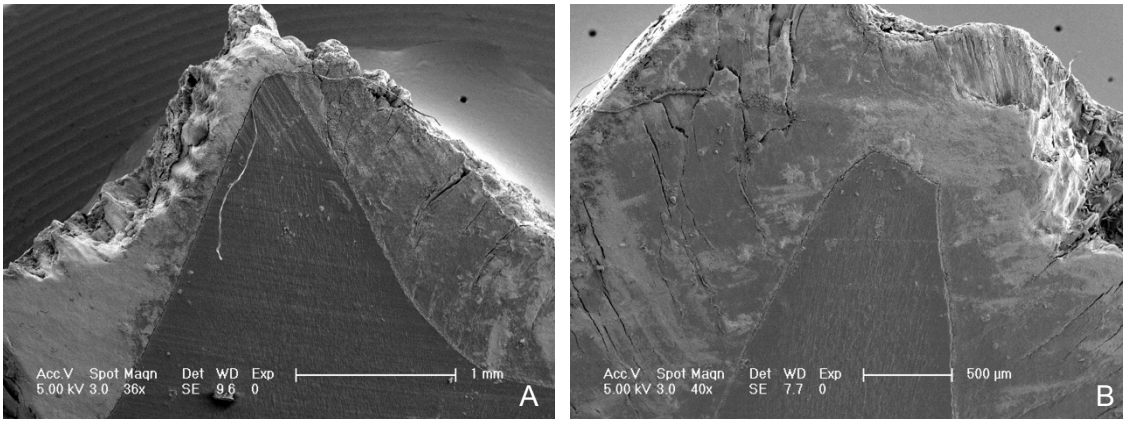

Figure 9-19 SEM images of the hypoplastic AI teeth show the rough and uneven enamel surface.

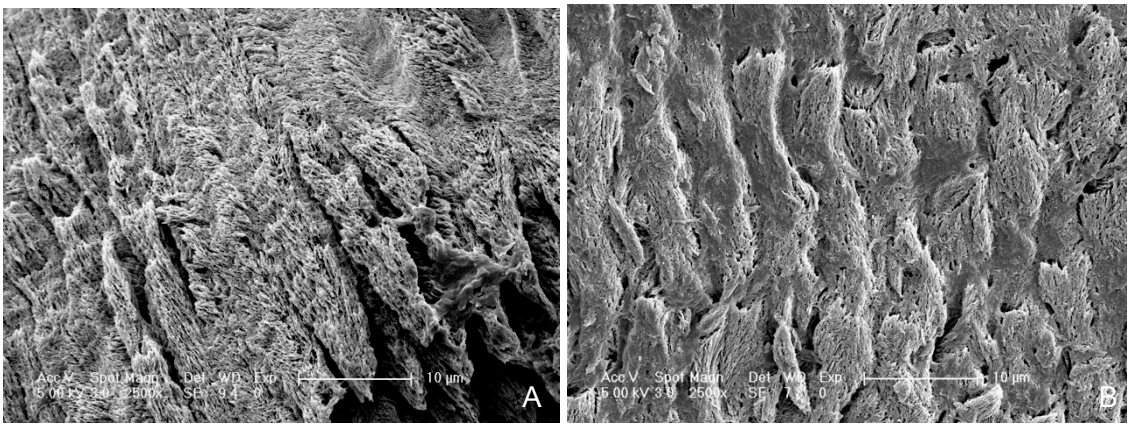

Figure 9-20 SEM images showing the disorganised enamel rod and crystals. A; The boundaries between each enamel rod are difficult to be distinguished. B; The individual crystals seem to have coalesced that leads to the 'glass-like' appearance.

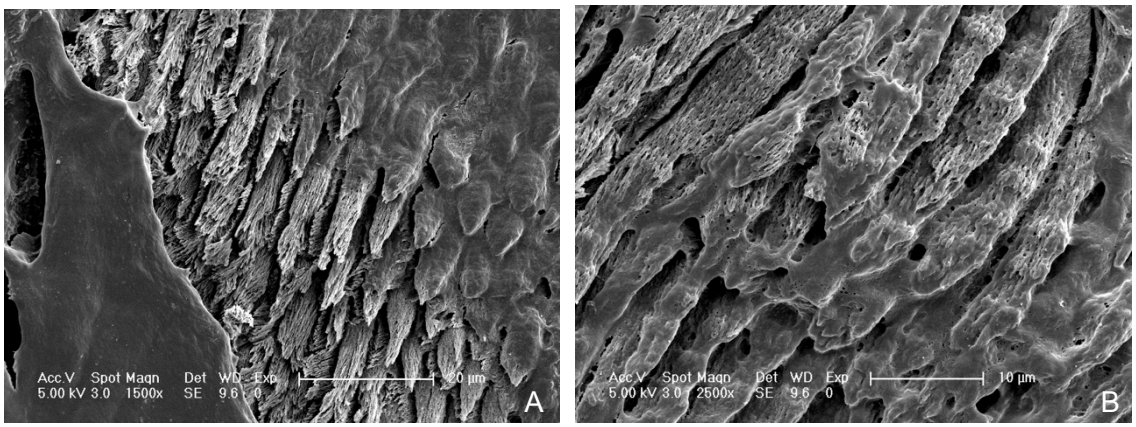

Figure 9-21 'Glass-like' structure covering the crystal.

9.4 Discussion

SEM imaging is a frequently used method to identify the morphological alteration of teeth. For SEM imaging, the enamel had to be etched and in this study 37% phosphoric acid was used.

The control teeth sample examined under SEM showed the typical appearance of enamel rod structure after being etched. From the image of the control teeth, it showed a distinctive etching pattern either Type I or II where the prism core was dissolved and leaving the enamel rod peripheries intact and peripheral region of the prism are dissolved preferentially and leaving the prism cores intact respectively (Mahoney et al., 2004a). The enamel rod orientation was continuous and direct without any interruption. HSB were present at the middle third of region of the enamel layer.

9.4.1 SEM for MIH teeth

In hypomineralised enamel, the typical enamel-etching pattern is absence and the enamel rod boundaries were not clearly demarcated (Mahoney et al., 2004a). In the MIH group, generally the enamel rods appear unorganised with interruption of the rods direction. Most of the affected enamel as described earlier was covered by a featureless and structureless layer (Jalevik et al., 2005). It is possible that etching a less organised enamel structure may not result in classic etch pattern. The reason may be due to poor acid solubility in less mineralized enamel, possibly due to the low mineral content and higher organic matrix as exhibited in the Raman spectra where the carbonate content increased in the hypomineralised enamel.

These findings are in line with a previous study where porous enamel contained higher carbon concentrations (Jalevik et al., 2005, Fagrell et al., 2010). Enamel in teeth affected by MIH exhibited disorganised enamel rods, a porous structure and loosely packed crystallites (Jalevik et al., 2005, Fagrell, 2011). However, carbonate substitution in hydroxyapatite crystallite is known to increase acid solubility of the mineral and should work in an opposite way (Weatherell, 1975). It is thought that the increased carbon depends on the amount of remaining organic matrix, as it has been suggested that protein might reduce the access of inorganic ions to crystallites (Robinson et al., 1971).

Hypomineralised enamel demonstrated an 8-21 fold higher protein content that might limit the action of the acid to the hydroxyapatite (Farah et al., 2010b). Consequently, the longer time required for enamel dissolution after etching may have some effect on bonding between restoration/adhesive and the affected first permanent molar enamel.

The basic enamel structure with enamel rods and inter rod zones was found in porous part of enamel as well as in the normal part but the packing of the hydroxyapatite crystals seemed to be looser and poorly organized in the porous part. When the clinical and histological appearance of MIH was compared with normal enamel in a polarisation microscope analyses, yellow／brown enamel opacities were shown to be more porous than lighter opacities (Da Costa-Silva *et al*. 2011).

In this study, the SEM appearance between white/cream, yellow/brown or PEB type of defect showed similar features in term of porosity, unorganised enamel rods and amorphous appearance due to the presence of the structureless layer. Most of the MIH teeth had been restored with different restorative materials. From the image at high magnification, it showed formation of a gap between the restorative material and the enamel, which could lead to micro leakage and failure of the restoration.

It is important for a dentist to choose the most suitable type of restoration depending on the severity of the tooth condition. Fissure sealant is a good choice of treatment for the mild cases where the enamel appears to be in good quality and clinically and also radiographically is caries free. In moderate cases, where the enamel and dentine is involved, composite restoration is the treatment of choice. However, for the teeth with PEB where breakdown possibility is high, the treatment is either restoration or extraction. If they choose to have restoration, stainless steel crown is the best option to prevent further enamel breakdown (Daly and Waldron, 2009).

Placement of the restorative material on a defective and brittle enamel as shown in this study is not helpful. It is proved from the SEM images that a gap was present between the tooth and the restoration that maybe due to continuous enamel breakdown as the FPM is subjected to the heavy occlusal load everyday. Hence, stainless steel crown can protect the tooth from further damage.

9.4.2 SEM for AI Teeth

In primary AI tooth, the SEM image is similar to the normal enamel microstructure with well-organised enamel prism and typical pattern of acid demineralisation.

It was a totally different finding in the permanent AI teeth. Those teeth diagnosed with hypocalcified type of AI showed a different layer in the enamel, which represented the level of mineralization. Macroscopically, the cut surfaces of the enamel indicated two layers of enamel. When these teeth were examined under SEM the outer layer appeared lighter than the inner layer. Apparently, the outer layer was more mineralized than the inner layer of the enamel. This finding resembled in the previous literature where the mineralization process starts at the incisal edge towards the cervical margin, following the growth of the tooth crown (Robinson et al., 1995). After demineralization with phosphoric acid, the outer layer of the enamel disclosed the pattern of etched enamel. However, the prisms appeared very porous and unorganized. The individual crystal also seemed to be fused to each other and that in normal teeth the crystal can be visualized clearly. It is supported by the previous study, which described the condition as ‘glass like’ appearance (Shore et al., 2010).

The inner layer of the enamel appeared denser when viewed at lower magnification. The finding was similar with the MIH group where the enamel prism was obscured by a structureless layer and probably due to the high organic matrix content that prevented the demineralization process by acid etching.

In a previous study, they found type I and type II etching pattern in hypomineralised AI after being etched with 35% phosphoric acid for 30 second (Sanchez-Quevedo et al., 2006). However, in this study, both patterns were not noted, which could be due to the difference in the dissection of the tooth sample. The enamel rod in hypomineralised AI have also been described as decussated pattern, superimposed filamentous pattern or

arcade-shaped outlined because these features were also present in normal developing human enamel and though to persist in immature morphological pattern as in AI (Sanchez-Quevedo et al., 2001).

In the hypoplastic type of AI, the enamel appeared thin with an irregular surface. The enamel was insufficient in quantity as well as quality. The enamel rods in hypoplastic AI have abnormal morphology and are irregular in their course. The enamel showed poorly organized rods and difficulty to establish the boundaries between each enamel rod compared to the unaffected enamel (Shore et al., 2010, Wright et al., 1991). Both studies focused on local and smooth hypoplastic AI respectively. In this study the hypoplastic AI teeth were as classified as rough hypoplastic, which can be seen from the low magnification, which showed the irregular, rough and variations of thickness. Porosity and ‘glass-like’ appearance were also present throughout the enamel thickness.

CHAPTER 10

SYNOPSIS

10 SYNOPSIS

DDE experience among young children has important clinical significance, as it can cause tooth sensitivity, tooth wear, aesthetic concern as well as predisposition to the development of dental caries (Lunardelli and Peres, 2005). These children undergo dental treatment as early as six years of age. Providing dental treatment in such early age is a challenge to the dentist. Optimal treatment should be established depending on the child's compliance, severity of the defect, occlusion, extend of the treatment required and long term prognosis of the affected tooth.

10.1 Sample Collection

In this study, the focus was towards determining the phenotypic characteristic in MIH and AI teeth. The MIH teeth obtained for this study were extracted due to their poor prognosis. The teeth were extracted either under local anaesthetic (LA) or general anaesthetic (GA). The decision to extract the teeth was achieved usually after discussion with the orthodontist. The primary investigator had to liaise with the SHOs and find out when the patients identified from the anomalies clinic were due to have the teeth extracted under GA, and then attend the GA to collect the teeth and return them to the lab as soon as possible to ensure appropriate storage.

However, AI teeth sample were quite difficult to obtain due to the rarity of the condition. Most of the AI patients were already in permanent dentition and treatment is to preserve the tooth structure for as long as possible. The extractions were due to poor prognosis and part of orthodontic treatment plan. All of the patients and parents were very happy to participate in this study, as they are also interested to know more about their child's teeth condition.

10.2 Phenotypic Characteristic

Table 10-1 indicates the summary for the phenotypic characteristic findings for MIH and AI teeth comparing with the control group. It consists of type of enamel defect, colour changes, hardness, chemical variation and ultrastructure.

10.2.1 Phenotypic Characteristic for MIH

Twelve MIH teeth from three patients were used in this study with different types of enamel defect from white/cream to PEB with abnormality in the translucency of the enamel (opacity) and sharp demarcation between the affected and sound enamel. This is in contrast to diffuse opacities found in enamel affected with fluorosis (Weerheijm et al., 2001). The surface initially develops with normal enamel thickness but easily broken down due to force from mastication (Weerheijm, 2004).

MIH can be confused with fluorosis and AI. It can be differentiated from fluorosis by demarcated opacity and by the structure of the enamel (fluorosis is caries resistant and MIH is caries prone) and fluorosis is directly related to the high fluoride intake (Weerheijm, 2004). The appearance of severe form of MIH can mimic the appearance of AI. In AI, the clinical presentation is usually associated with a family history.

In this study, it was found that the severity of teeth affected with MIH had a relationship with the colour of the enamel opacity where the white/cream lesion showed higher ΔE and hardness (VHN) value and lower carbonate to phosphate ratio compared to the yellow/brown and PEB. This finding is in line with a previous study where the yellow/brown opacities were at higher risk for PEB than the white ones (Da Costa-Silva et al., 2011). The presence of atypical restorations also could be the indicator of the severity of the lesion (Da Costa-Silva et al., 2011).

Histologically, the enamel structure showed a great porosity, unorganised enamel rod, and presence of amorphous structureless layer covering the enamel rod, which seemed resistant to the acid etching that also been described in the earlier study (Suckling et al., 1989, Crombie et al., 2013, Fagrell et al., 2010, Jalevik et al., 2005). Based on the histological findings, it has been postulated that the ameloblasts might have been affected during the early maturation stage of amelogenesis but there are also possibility that the cells have been affected since the secretory stage. Theoretically, health problem during enamel mineralisation could have disturbed the ameloblastic activity (Jalevik et al., 2001b).

The porous subsurface enamel structure may promote bacteria penetration into the dentine which leads to inflammation of the pulp and complicating the use of LA and more susceptible to caries formation than the teeth without such defect (Lygidakis, 2010, Leppaniemi et al., 2001).

Restoring MIH teeth is considered as a big challenge faced by many clinicians. Apart from the restoration difficulties, behaviour problems associated with dental fear and anxiety can be an issue when treating children with MIH. This behaviour can be due to pain experienced during multiple treatments appointment as many of them had inadequate anaesthesia or had treatment without LA (Jalevik and Klingberg, 2002).

It could be interesting to include adhesion properties in hypomineralised enamel in future studies as a result of defective enamel. Two approaches had been proposed to restore the hypomineralised teeth either removal of all defective enamel until sound surface reached or removal of the porous enamel only until resistance to the bur or probe felt (Lygidakis, 2010). The first approach results in the loss of a lot of tooth structure but better if an adhesive material is to rely upon bonding to enamel. The second approach is less invasive, but it leads to continuation of the defective enamel to break down.

Another study recommends additional procedure to enhance the adhesion, with pre-treatment of the enamel with 5% sodium hypochlorite to remove extrinsic proteins encasing the hydroxyapatite and therefore facilitate etching and resin penetration (Mathu-Muju and Wright, 2006). This recommendation is justified with the recent study that found higher protein content (15 – 21 fold) in MIH teeth than the sound enamel (Farah et al., 2010b). The increase of the protein content might limit the access of acid to the hydroxyapatite crystallites.

10.2.2 Phenotypic Characteristic for AI

Seven AI teeth from three patients were included in the study; a primary incisor, two teeth from a patient with hypoplastic AI (rough) and four teeth from a patient with hypocalcified AI. In contrast with MIH, the enamel defect in AI is more diffuse and affecting the whole surface of the crown of the tooth. However, the primary tooth has no enamel defect.

Clinically, the hypoplastic AI showed a reduction in the enamel thickness with very rough surface. Some of the areas are nearly exposing the dentinal layer. The colour changes in hypolastic AI were similar to the hypocalcified AI since the lesion was more diffuse and not well demarcated. However, hardness values in the hypoplastic enamel were lower than the hypomineralised teeth. Previous study showed that enamel

hardness in the pitted hypoplastic is comparable to the healthy enamel, while the smooth hypoplastic revealed very low hardness (Pavlic et al., 2007b). The difference could be possibly due to the presence of both hypoplastic and hypomineralised enamel defect in a tooth.

The hypocalcified AI teeth presented with diffuse yellow/brown enamel discolouration. The enamel looked intact. However, the hardness of enamel was significantly lower than the normal healthy enamel as shown in the previous study (Faria-e-Silva et al., 2011) and might be explained by the lower mineral content. The increase of the carbonate to phosphate ratio in hypoplastic and hypocalcified teeth reflect the metabolic activity of the ameloblasts; that is, high metabolic rate corresponds to high carbonate whereas low metabolic rate corresponds to low carbonate levels. The recent study showed marked carbon content in hypocalcified AI (El-Sayed et al., 2010).

Histologically, the hypoplastic AI showed thinning of the enamel layer with rough and irregular surface. The crystallite in the enamel rod showed disorganized arrangement and presence of 'glass-like' appearance covering rods as described by Shore et al. (Shore et al., 2010). This appearance also has been described as laminated appearance (Pavlic et al., 2007a).

The hypocalcified AI teeth in this study showed two distinct layers in the enamel. Macroscopically the outer layer appears lighter than the inner layer that looked darker and denser. After the etching with 37% phosphoric acid, the outer layer presented with porous enamel rods. Whereas, the inner part of the enamel seemed unaffected by the etching process when the enamel rod obscured by an abnormal amorphous material as described in the earlier study (Faria-e-Silva et al., 2011). These characteristic might explained the lower bond strength and deproteinisation process using sodium hypochlorite is proposed to improve the bond strength. However, it does not have significant effect on the bond strength to enamel or dentine (Faria-e-Silva et al., 2011).

Group	Type of defect	Colour (ΔE)	Mean Hardness Value (VHN)	Mean Carbonate to phosphate ratio	SEM and Radiographic findings
Control	Normal	1.12 – 5.93	311.68 (± 61.65)	0.035 (± 0.006)	Homogenous arrangement of enamel rod Presence of typical appearance of demineralized enamel Radiograph: Difference in radiodensity between enamel and dentine separated by well defined DEJ
MIH	White/cream Yellow/brown PEB	2.57 – 7.76 3.82 – 24.48 6.22 – 21.37	86.11 (± 13.57) 57.26(± 7.80) 41.64 (± 4.06)	0.070 (± 0.030) 0.135 (± 0.042) 0.112(± 0.009)	Disorganised enamel rod Porous Presence of structureless layer covering the enamel rod Radiograph: Findings similar to caries, radiolucent at the affected site depending on the depth of defect
AI	Normal (primary) Yellow/brown Hypoplastic with missing enamel	1.34 5.24 – 7.58 5.50 – 7.87	242.58 (± 65.76) 108.21 (± 13.74) 27.34 (± 2.34)	0.045 (± 0.002) 0.052 (± 0.015) 0.071 (± 0.003)	Hypocalcified AI Presence of 2 distinct layer of enamel with porous outer layer and enamel rod in the inner layer covered with 'glass-like' appearance structure Radiograph: Similar radiodensity between enamel and dentine, presence of taurodontism at molar teeth Hypoplastic AI Thin enamel with irregular and rough surface Irregular arrangement of crystallites Indistinct enamel rod boundaries when one enamel rod combined with the adjacent rod Presence of 'glass-like appearance when individual crystals seemed to have fused each other Radiograph: Smaller teeth size, absent of proximal contact, reduce enamel thickness,

Table 10-1 Summary of the phenotypic characteristic of MIH and AI group compared to the Control group

11 CLINICAL RELEVANCE

MIH and AI are two different types of enamel defects. MIH basically occurs when a disturbance occurs during the development of the tooth and is influenced by various factors within the prenatal, perinatal and postnatal period. AI is a hereditary enamel defect, which also affects tooth formation.

Both conditions may share the same clinical appearance and it can be difficult to confirm the diagnosis especially if the lesion in milder form, or the patient presents in the early mixed dentition. Usually MIH occurs in an asymmetrical manner and is related to the first permanent molars and also the permanent incisors as these are teeth that are form earlier. However, AI usually presents in a more symmetrical way and can affect any teeth. It is usually also associated with similar family history among family members.

In dentistry, both conditions give a challenge for the dentist not only to make a provisional diagnosis but also the management in the short and long term. The enamel defect can be detected as soon as the tooth erupts, which can be as early as 6 years old for the permanent teeth. The treatment should consider the main complaint of the patient, severity and cooperation to undergo the treatment. Therefore, in order to provide the best treatment, it is important to understand the pathogenesis of each condition to enhance a better knowledge before any treatment given.

In this study the DDE index was used to record and describe the lesions. Even though it is a bit time consuming particularly if lesions involve several teeth, but it is a good method to describe the defect. The DDE index also helps when we want to correlate with the other phenotypic properties and assist diagnosis.

One of the main concerns in MIH and AI is aesthetics, if the permanent incisors are affected. MIH may not be as severe as AI if the lesion is confined to the first permanent molars. Patients with generalised discolouration of the enamel, can present with significantly affected self-esteem and also quality of life. The use of the spectrophotometer helped us in quantify the degree of discolouration in the enamel. Spectrophotometer is quite practical for daily clinic usage especially for anterior teeth but maybe slight difficult for the posterior teeth due to the size of the instrument.

The enamel hardness in MIH and AI teeth showed a significant decrease when compared to normal enamel. The hardness related to the type of defect where the PEB lesion showed the lowest hardness value in MIH group; while in AI group hypoplastic AI with missing enamel indicated the lowest. It is difficult to consider measuring hardness of the enamel as a tool to assist in making diagnosis unless if there is any device that can be used in oral cavity. However, based on the relationship between the enamel hardness and the colour of the enamel defect, it does show that the colour differences can be a basic parameter to aid the diagnosis and also the future prognosis of the teeth without measuring the hardness.

Raman spectra helps us to understand the particular content in normal and defective enamel. During early formation of enamel, the matrix content is higher than the mineral content. After the enamel starts the phase of maturation where the mineralisation take place, most of the organic content is reduced and there is a gradient decrease of mineral concentration from the surface towards DEJ. Increase carbonate to phosphate ratio in MIH and AI group showed the reduction in the quality of the enamel.

Images from the SEM proved that the prism in defective enamel seems to be disorganized, very porous and covered by an amorphous and structureless layer that postulated to be organic structure such as protein. Previously, it was suspected that this is the reason of failure of bonding to the restorative material as the etching cannot demineralise the enamel in MIH or AI, compared to normal enamel. Or it was thought that longer etching time or greater concentrated of etch was required.

From this study, all of the phenotypic characteristic that have been observed can help us to understand more about the behaviour of each enamel defect and anticipate the future outcome for each tooth. The relationship between the type of enamel defect and the other studied phenotypes may additionally support the dentist in managing children with MIH and AI.

CHAPTER 12
CONCLUSION

12 CONCLUSION

The different type of the enamel defects correlated with the differences in colour changes using measurements with the spectrophotometer. White/cream opacities show lower ΔE values compared to the other types of defect. Yellow/brown opacity show high values that can be observed clinically.

These findings also corresponded to the significant reduction of enamel hardness where the hypoplastic AI teeth showed the lowest hardness values. Difficulty to stabilise the tooth samples due to the convexity of the crown was the contributing factor that leads to the variation of measurement (increased standard deviation).

Chemically, the hypomineralised enamel had higher carbonate to phosphate ratio that caused instability of the microstructure of the enamel. Both hypocalcified and hypoplastic AI teeth in this study showed a significant increase in the carbonate to phosphate ratio. This finding is not in agreement with a previous study, which showed no difference in carbonate content between AI and the normal enamel. This could be due to the application of different techniques and instruments.

In the MIH teeth, the ultrastructure indicated disorganised, porous, loss of enamel rod structure and the presence of structureless layer that seems to be unaffected during the demineralisation process with phosphoric acid covering the enamel rod. The findings were quite similar between the white/cream, yellow/brown and PEB type of defect. However, the structure in hypocalcified AI was different where the enamel has two distinct layers. The enamel rods could be distinguished in the outer layer but with greater porosity. The inner layer looked denser and was covered with a ‘glass-like’ appearance that could be unaffected by the phosphoric acid. The structure for hypoplastic AI was quite similar to MIH; porosity, disorganised enamel rod and loss of enamel rod structure.

CHAPTER 13
FUTURE WORK

13 FUTURE WORK

Correlation of clinical phenotype with the genotype will be extremely valuable for managing patients with enamel defects. Moreover, this knowledge allows us to make better predictions of affected patients and manage or prevent their related problems.

The results from the study, provide a general view of the phenotypic characteristic of the enamel in MIH and AI teeth. Limitations in this study includes the difficulty to find samples, especially the AI teeth, leading to a small sample used in the study, and making it difficult to make comparisons It would be good if all types of AI; hypoplastic, hypocalcified and hypomature were included in the study. Genetic input from each AI patient also could be interesting in order to understand both the phenotype and genotype properties for each patient.

Future work will demand more participants to be involved in the study to ensure a larger tooth sample size is achieved. However, the need for extraction of these teeth is depending on the severity as most of the patients need to keep the teeth as long as possible before any permanent future management can be carried out. Thus, the sample collection is restricted to extraction as part of the treatment or very poor prognosis teeth. It would be useful if in future study can include fluorosis as one of the intervention group as this condition also affect the patient aesthetically.

In order to understand more about the phenotypic characteristic of these conditions, it may be interesting to use an advance imaging technique such as optical coherence tomography (OCT) as this method does not need any sample preparation to obtain new information from the defective enamel.

Most dentists have experienced difficulty bonding to restorative materials, due to the limitation in etching the enamel. Further study regarding the bonding properties between the enamel and the current restorative material could help the dentist to find the best choice of material to treat these teeth.

Wallace indenter was used in this study to measure the enamel hardness is quite challenging in order to obtain the most stable surface before the indentation can be made. This is due to the bulbosity of the tooth surface that made the tooth very unstable. It is wise to think about other means to measure the enamel hardness so that

the result is more accurate and consistent. Nano indentation with atomic force microscopy (AFM) may produce a better result.

The other instrument that can be used to measure enamel hardness is BioDent™. It is a bench-top Reference Point Indentation platform that enables the measurement of material properties of tissues and biomaterial. The in vitro to in vivo testing modes set BioDent™ apart from other instruments, enabling the acquisition of previously impossible material property data that has clinical relevance. The enamel hardness could possibly be measured intraorally without the needs of extracting the tooth and maybe aid the diagnosis process.

CHAPTER 14
SCIENTIFIC DISEMINATION

14 SCIENTIFIC DISEMINATION

In preparation for submission December 2014

Tittle: Phenotypic Properties Of Enamel In Molar-Incisor Hypomineralisation (MIH) and Amelogenesis Imperfecta (AI) Teeth

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Journal: Journal of Dental Research (JDR)

14.1 Presentation

Poster presentation at International Association of Dental Research (IADR) in Cape Town, South Africa in June 2014 as shown on the next page.

Phenotypic properties of enamel in Molar-Incisor Hypomineralisation and Amelogenesis Imperfecta

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Introduction

Molar incisor Hypomineralization (MIH) can be defined as qualitative defect of systematic origin of the enamel, involving one or more first permanent molar, which is frequently associated with affected incisors.[1] **Amelogenesis Imperfecta** (AI) is an heritable condition, affecting the structure and clinical appearance of the enamel of all or nearly all the teeth in a more or less equal manner. This condition may also be associated with morphological or biological changes elsewhere in the body.[2] Characterisation of the phenotypic properties of both these conditions is essential to predict their severity and also to provide novel information towards adequate treatment plan for each individual patient affected.

Aims

The aim of this study is to characterize the phenotypic properties of MIH and AI teeth. The objectives were set to assess: tooth colour (on and off the anomaly), hardness (on and off the anomaly) and the ultrastructure of the affected enamel vs. normal enamel.

Materials and methods

Ethical approval was obtained. Twelve control, twelve MIH and six AI teeth were collected. The phenotype (DDE Index) for each sample was recorded. Prior to characterisation, the teeth were debrided and stored in 0.1% thymol at 4ºC. Colour was examined using a spectrophotometer (SpectroShade™ Micro) to quantify the variation in colour defined as ΔE. Wallace indentation (H.M Wallace, Croydon, England) was also performed on the samples to assess the hardness value, denoted VHN (Vickers Hardness Number). Finally, the samples were sectioned at specific affected sites to image the ultrastructure of the enamel layer using a scanning electron microscopy (FEI XL30 FEGSEM (FEI UK, UK) .

Results

a)Type of Defects

DDE index was used to record the lesion based on the location, extension, type, and severity of the defect for each sample. Table 1 indicates the type of defect for the MIH and AI teeth.

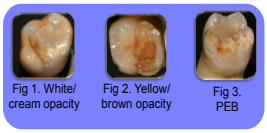


Fig 1. White/ cream opacity | Fig 2. Yellow/ brown opacity | Fig 3. PEB

The teeth affected with MIH had different type of enamel defect; white/cream, yellow/brown and post-eruptive breakdown (PEB). All of the teeth were extracted from three patient where each patient had contribute four first permanent molars.

Tooth Sample	Type of defect
MIH 1	White/cream
MIH 2	Yellow/brown
MIH 3	Yellow/brown
MIH 4	White/cream
MIH 5	White/cream
MIH 6	White/cream
MIH 7	PEB
MIH 8	PEB
MIH 9	Yellow/brown
MIH 10	PEB
MIH 11	White/cream
MIH 12	White/cream
AI 1	Yellow/brown
AI 2	Yellow/brown
AI 3	Yellow/brown
AI 4	Yellow/brown
AI 5	Hypoplastic (missing enamel)
AI 6	Yellow/brown

Table 1: Type of defect for MIH and AI teeth sample

Fig 4. Hypoplastic with missing enamel | Fig 5. Yellow/ brown opacity

Two of the AI sample were extracted from a patient with hypoplastic type of AI and the other four were from hypomineralised type of AI. The teeth were either yellow/brown discolouration or hypoplastic with missing enamel.

b) Colour

Delta E value between 0 and 2 is considered to be excellent match, 2 and 4 is a good match and any Delta E above 4 is borderline as the colour difference between the tooth and the standard library shade is detectable by human eye. The MIH and AI group showed Delta E of 2.57 for white/cream lesion and as high as 24.48 in yellow/brown discolouration (Fig. 6).

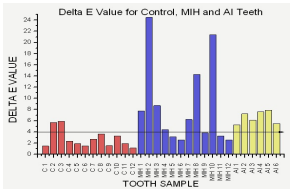


Fig. 6 Delta E value for the Control, MIH and AI teeth. The values above 4 are detectable by human eye.

c) Hardness

Mean VHN value for enamel in control teeth is 313.84 (95% CI; 296.78 to 330.90). However, the mean VHN for the MIH and AI are significantly lower than the control teeth (p value = 0.004) as shown in Fig. 7 which indicate how fragile is the enamel with poor mineralisation

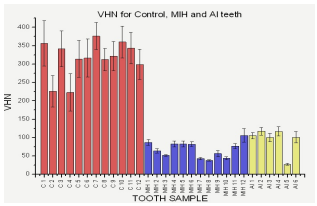
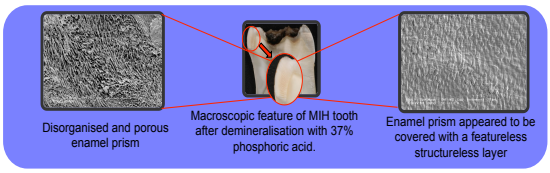


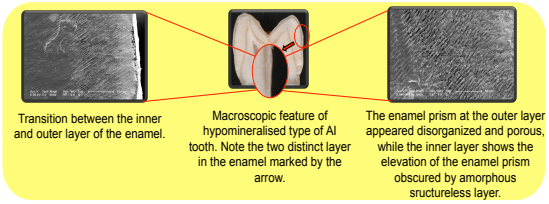
Fig. 7 The enamel hardness for Control, MIH and AI teeth.

d) Ultrastructure

SEM imaging revealed the enamel microstructure. The MIH and AI enamel prism showed disorganised, porous with some areas of the covered with structureless layer.



Disorganised and porous enamel prism | Macroscopic feature of MIH tooth after demineralisation with 37% phosphoric acid. | Enamel prism appeared to be covered with a featureless structureless layer



Transition between the inner and outer layer of the enamel. | Macroscopic feature of hypomineralised type of AI tooth. Note the two distinct layer in the enamel marked by the arrow. | The enamel prism at the outer layer appeared disorganized and porous, while the inner layer shows the elevation of the enamel prism obscured by amorphous structureless layer.

Discussion

- Spectrophotometry described the colour of the lesion in an objective way compared to visual assessment. [3] The colour changes may reflect the differences in the hardness and its mineral content.
- In MIH the reduction in hardness related to the type of defect. It has been suggested that the yellow/brown lesion as a more severe form of MIH and more likely to develop PEB.[4]
- Both MIH and AI showed unorganized enamel prism with interruption of the prism direction, porous and covered by a featureless and structureless layer. The defective ultrastucture can contribute to the bacterial adhesion and further development of caries lesion possible cause of hypersensitivity.

Conclusions

The phenotypic properties of enamel in MIH and AI teeth showed higher Delta E value, low hardness and defective ultrastucture and vary according to the morphological properties.

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CHAPTER 15
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15 REFERENCES

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Dr Laurent Bozec

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If you need a large print, audio or translated copy of this document, please contact us on 020 3456 1067. We will try our best to meet your needs.

If you wish to discuss this study with a member of the research team or an independent expert who is not part of the research team, please ask Dr Susan Parekh

Thank you for taking the time to read this leaflet.

University College London Hospitals NHS
NHS Foundation Trust

A study of the genetics and the physical properties of dental anomalies

Publication date: 07/07/11
Date last reviewed:
Version number **2**

Parent Information Leaflet

A Study of the genetics and the physical properties of dental anomalies

Invitation

Your child is being invited to take part in a research study. Before you make a decision, it is important that you know why the research is being done and what it would involve from your child. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if anything is not clear at any time before or after participating. If you need more information we are willing to spend more time to satisfy you before taking any decision.

What is the purpose of the study?

To gather more information about dental anomalies, such as enamel defects (Amelogenesis Imperfecta), and dentine defects (Dentinogenesis Imperfecta). We want to use this information to improve our knowledge of genetics and how they affect the properties of the teeth.

Why has my child been chosen?

We are asking all patients who have been diagnosed with dental anomalies and members of their families with the same or other dental conditions to participate in the study

Does my child have to take part?

No. It is up to you to decide. If you do decide to participate we will ask you to sign a consent form. If you, or your child, change your mind, you are free to withdraw at any time, without giving a reason. The standard of care your child receives will not be affected in any way.

What will happen to me if my child takes part?

We will ask you and your child some questions about your child's teeth, take photographs, and a saliva sample. The saliva sample will be used to link your child's DNA with the physical properties of their teeth. We will also measure the colour of the front teeth using a machine called the spectroshade™ micro, which rests gently on the teeth and uses a light to record the shade of the tooth (see information sheet provided). If any teeth need to be extracted as part of your child's treatment, these will be collected for laboratory testing of the teeth. Your child will not need to do anything else. If any member of your family has similar teeth, we will invite them to take part as well, as this will help to detect the common dental genes in families. If you do not want other members of your family to participate, you can refuse and your child's treatment will not be affected in any way.

What are the possible disadvantages or risks of taking part?

There are no risks anticipated. None of your answers will affect your treatment in any way.

What are the possible benefits?

We cannot promise the study will help you, but the information we get might help treat young people with dental anomalies in the future.

What will happen with the results?

Any samples that we collect will be stored using a study ID number, so that they cannot be directly linked to your child. We hope to publish the results of the study on completion.

Will taking part in the study remain confidential?

Yes. We will keep your information in confidence. This means we will only tell those who have a need or right to know. The safety and security of the data will be the responsibility of the principal investigator (Dr Susan Parekh). The information will also be stored in a database developed by Strasbourg University (phenodent database), who we work closely with. All information will be anonymised before putting on the phenodent database.

What happens if something goes wrong?

In the event that something does go wrong and you are harmed during the research and this is due to someone's negligence then you may have grounds for a legal action for compensation against UCLH NHS Trust, but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you (if appropriate).

Who has reviewed the study?

All research in the NHS is looked at by independent group, called a Research Ethics Committee to protect your safety, rights, wellbeing and dignity. This study has been reviewed and given favourable opinion by the Joint Research Ethics Committee. Thank you for reading this, please ask any questions if you need to.

Research team:

Dr Susan Parekh,

Dr Agnes Bloch Zupan,

Dr Peter Brett,

Dr Laurent Bozec

Mashael Abdullatif

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If you wish to discuss this study with a member of the research team or an independent expert who is not part of the research team, please ask Dr Susan Parekh

Thank you for taking the time to read this leaflet.

University College London Hospitals NHS
NHS Foundation Trust

A study of the genetics and the physical properties of dental anomalies

Publication date: 08/08/11

Date last reviewed

Version number **3**

Patient's Information Leaflet

A Study of the genetics and the physical properties of dental anomalies

Invitation

You are being invited to take part in a research study. Before you make a decision, it is important that you know why the research is being done and what it would involve from you. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if anything is not clear at any time before or after participating. If you need more information we are willing to spend more time to satisfy you before taking any decision.

What is the purpose of the study?

To obtain and gather more information about dental anomalies, such as Enamel defects (Amelogenesis Imperfecta AI), and dentine defects (Dentinogenesis Imperfecta DI). We want to use this information to improve our knowledge of genetics and the properties of the teeth, to provide better support and long term care.

Why has I have been chosen?

We are asking all patients who have been diagnosed with dental anomalies and members of their families with the same or other dental conditions to participate in the study

Do I have to take part?

No. It is up to you to decide. If you do decide to participate we will ask you to sign a consent form. If you change your mind, you are free to withdraw at any time, without giving a reason. The standard of care you will receive will not be affected in any way.

What will happen to me if I take part?

We will ask you some questions about your teeth and your medical history, and examine your teeth, take photographs, and a saliva sample. The saliva sample will be used to link your DNA with the physical properties of your teeth. If you require any teeth to be extracted as part of your treatment, these will be collected for laboratory testing of the teeth. You will not need to do anything else. If any member of your family has similar teeth, we will invite them to take part as well, as this will help to detect the common dental genes in families. If you do not want other members of your family to participate, you can refuse and your treatment will not be affected in any way.

What are the possible disadvantages or risks of taking part?

There are no risks anticipated. None of your answers will affect your treatment in any way.

What are the possible benefits?

The information from this study will hopefully be used to help us expand our knowledge about the genetics of dental anomalies, and relate this to the appearance of the teeth, identify affected families and provide better support and treatment.

What will happen with the results?

Any samples that we collect will be stored using a study ID number, so that they cannot be directly linked to you. We hope to publish the results of the study on completion. All confidential information will be coded and you will not be identifiable in any way.

Will my taking part in the study remain confidential?

Yes. All information that is collected about you during the research will remain strictly confidential and will be seen only by the investigators named on this sheet. The safety and security of the data will be the responsibility of the principal investigator (Miss Susan Parekh). This information will be recorded in such a way that it is completely anonymous and you cannot be individually identified in anyway.

The information will also be stored in a database developed by Strasbourg University (phenodent database), who we work closely with. All information will be anonymised before putting on the phenodent database.

Who has reviewed the study?

All research in the NHS is looked at by independent group, called a Research Ethics Committee to protect your safety, rights, wellbeing and dignity. This study has been reviewed and given favourable opinion by the Joint Research Ethics Committee. If you would like to see a summary of the findings from the study when it is completed, please tell Miss Parekh or any of the other dentists you see.

18 APPENDIX 3

University College London Hospitals NHS
NHS Foundation Trust

The Eastman Dental Hospital
256 Gray's Inn road
London
WC1X 8LD

Telephone: 020 3456 - 7899
Direct Line: 020-3456 - 1067
Fax: 020-3456-2329
Web-site: www.uclh.nhs.uk

Version 1
Study Number:....
Patient Identification Number for this trial: .....

PARENT CONSENT FORM

Title of Project:

A Study of the genetics and the physical properties of dental anomalies.

Name of Researchers: Dr Susan Parekh, Dr Agnes Bloch-Zupan, Dr Peter Brett, Dr Laurent Bozec, Miss Amanda O'Donnell, Mashael Abdullatif, Nurjehan Mohamed Ibrahim and Nabilah Narith.

Please initial box

1. I confirm that I have read and understood the information sheet dated 21/12/10 (version 1) for the study. I have been allowed some time to think about this, ask questions, and have had these answered in a way that I understand. ☐

2. I understand that my child's is voluntary and that I am free to withdraw at any time, without giving any reason , without their medical care or legal rights being affected. ☐

3. I understand that sections of any medical notes may be looked at by the researchers and responsible individuals from regulatory authorities where it is relevant to my child taking part in research. I give permission for these individuals to have access to my child's records. ☐

4. I give permission to the investigators to pass clinical data collected from my child's examination to my General Practitioner or General Dental Practitioner ☐

5. I understand that the samples taken from my child may be stored and used for the purpose of further research at a later date. I understand that these results will also remain anonymous. ☐

6. I understand that (this project or future research) will include genetic research aimed at understanding the genetic influences on dental defects in children. ☐

7. I agree for my child to take part in the above study. ☐

Name of Patient	Date	Signature of parent
Name of Person taking consent	Date	Signature

When completed, 1 for patient; 1 for researcher site file; 1 (original) to be kept in medical notes

UCL HOSPITALS

UCL Hospitals is an NHS Foundation Trust comprising: The Eastman Dental Hospital, The Heart Hospital, Hospital for Tropical Diseases, National Hospital for Neurology and Neurosurgery, The Royal London Homoeopathic Hospital and University College Hospital (incorporating the former Middlesex and Elizabeth Garrett Anderson Hospitals).

S Parekh Version 1

For further information about this study please contact Dr Susan Parekh

Phone : 020 3456 1067 email: s.parekh@eastman.ucl.ac.uk

UCLH welcomes feedback from their patients who have been involved in research. In the first instance, you should inform the Principal Investigator. If you are not satisfied with the response of the research team then you should address your complaints to the UCLH complaints manager at UCLH postal address or through our website http://www.uclh.nhs.uk/Contact+us/. To help us identify the research study you have been involved in, please mention the title and the name of the research doctor or principal investigator. You can find this information on the Patient Information Sheet.

19 APPENDIX 4

University College London Hospitals NHS
NHS Foundation Trust

The Eastman Dental Hospital
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Telephone: 020 3456 7899
Direct Line: 020-3456-1067
Fax: 020-3456-2329
Web-site: www.uclh.nhs.uk

Version 1
Study Number:....
Patient Identification Number for this trial: .....

PATIENT CONSENT FORM

Title of Project:

A Study of the genetics and the physical properties of dental anomalies.

Name of Researchers: Dr Susan Parekh, Dr Agnes Bloch-Zupan, Dr Peter Brett, Dr Laurent Bozec, Miss Amanda O`Donnell, Mashael Abdullatif, Nurjehan Mohamed Ibrahim and Nabilah Narith.

Please initial box

1. I confirm that I have read and understood the information sheet dated 21/12/10 (version 1) for the study. I have been allowed some time to think about this, ask questions, and have had these answered in a way that I understand. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason , without my medical care or legal rights being affected. ☐

3. I understand that sections of any medical notes may be looked at by the researchers and responsible individuals from regulatory authorities where it is relevant to my part in the research. I give permission for these individuals to have access to my records. ☐

4. I give permission to the investigators to pass clinical data collected from my examination to my General Practitioner or General Dental Practitioner ☐

5. I understand that the samples taken from me may be stored and used for the purpose of further research at a later date. I understand that these results will also remain anonymous. ☐

6. I understand that (this project or future research) will include genetic research aimed at understanding the genetic influences on dental defects in children. ☐

7. I agree for to take part in the above study. ☐

Name of Patient	Date	Signature of patient
Name of Person taking consent	Date	Signature

When completed, 1 for patient; 1 for researcher site file; 1 (original) to be kept in medical notes

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20 APPENDIX 5

Consent form for the Phenodent database

You/ your child have been asked to participate in the database project entitled "Diagnosing Dental Defects database D [4] / Phenodent.

The establishment of this registry has received the favorable opinion of CCTIRS 11.09.2008, and authorization of the CNIL on 18/05/2009 (Registration No. 908416).

I can at any time obtain additional information from Miss Susan Parekh (primary investigator) or Prof. Agnes Bloch-Zupan, Project Manager, the Reference Centre of dental manifestations of rare diseases, Department of Oral Health Care, University Hospital Strasbourg, Hôpital Civil, 1 place Hospital, F-67000 Strasbourg Cedex France or email: agnes.bloch@chru-strasbourg.fr

I authorize the registration of anonymous data and pictures in the database yes ☐ no ☐
and my ethnic background (via the collection of country and city of birth) yes ☐ no ☐
This information may also be used for teaching purposes

For data files, I authorize the possible dissemination of all images, yes ☐ no ☐
or only intraoral pictures yes ☐ no ☐

YOUR AGREEMENT TO PARTICIPATE IN THIS REGISTRY
My signature certifies that I clearly understood the information regarding my participation in this registry

Name of Patient	Date	Signature
Name of Parent	Date	Signature
Name of Person taking consent	Date	Signature

This document is to be performed in two original copies:
A copy kept by the person giving consent (or by the holders of parental authority if minor)
The other copy to be kept by the primary investigator, Miss Susan Parekh

21 APPENDIX 6

Dental anomalies proforma

study ID:.....

Date of clinic:..... Pt sticker:

Clinician name:.....

Ethnicity: White Mixed Black Asian Chinese Other.....

Referred by: GDP CDS HDS GP Other:.....

c/o: Nil pain sens appearance Other:.....

Relevant medical history:.....

.....

Fluoride history: supp Y/N water Y/N toothpaste child/adult

Dental history: restn Y/N ext Y/N LA Y/N sed Y/N GA Y/N

Family history (inc family tree): Plaque score:

Extra-oral features: Skeletal pattern I II III

Hair: normal/sparse skin:.....

face:..... hands/nails:..... Other:.....

Intra-oral features: lips gingiva palate mucosa saliva

Teeth present (chart):

Eruption: early Y/N delayed Y/N infraoccluded Y/N impacted Y/N

General/local Mild/mod/sev; teeth:..... Teeth:.....

Occlusion: Class I Class II i ClassIIii ClassIII OJ = OB: complete / incomplete

AOB Y/N

Dentine:

discoloured: Y/N abscess: Y/N tooth wear: mild / mod / sev (which teeth):

Enamel:

18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
DDE index: Location (L): 1 incisal ⅓; 2 gingival ⅓; 3 whole surface. Demarcation of defect (D): 1 demarcated; 2 diffuse; 3 both			55	54	53	52	51	61	62	63	64	65	Extent of defect(E): 1 < ⅓; 2 ⅓ – ⅔; 3 at least ⅔. Wear: mild mild; sev severe		
			85	84	83	82	81	71	72	73	74	75			
48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	37

Type of defect: 0 normal; 1 opacity (white/cream); 2 opacity (yellow/brown); 3 hypoplasia (pits); 4 hypoplasia (horizontal grooves); 5 hypoplasia (vertical groves); 6 hypoplasia (missing enamel); 7 discoloured enamel (not assoc. with opacity); 8 post-eruptive breakdown; 9 other defects;

Number / form / size:

18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
			55	54	53	52	51	61	62	63	64	65			
			85	84	83	82	81	71	72	73	74	75			
48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	37

con conical; shov shovel; dbl double; rog rounded or bulbous; tap tapered; cet talon cusp; can abnormal cusp; noc notched; mic microdont; mac microdont; inv invagination; env evagination; mih enlarged mamelons; pem enamel pearls; sup supernumerary; hyp hypodontia

Radiographic findings: taurodont Y/N thin enamel Y/N short roots Y/N
pulp stones Y/N apical area Y/N resorption Y/N

Diagnosis:

Proposed treatment plan:

1.
2.
3.
4.

Treatment to date:

Allocated to:

Review on anomalies clinic: Y/N when?

Photographs Y/N **saliva** Y/N **Consent** Y/N